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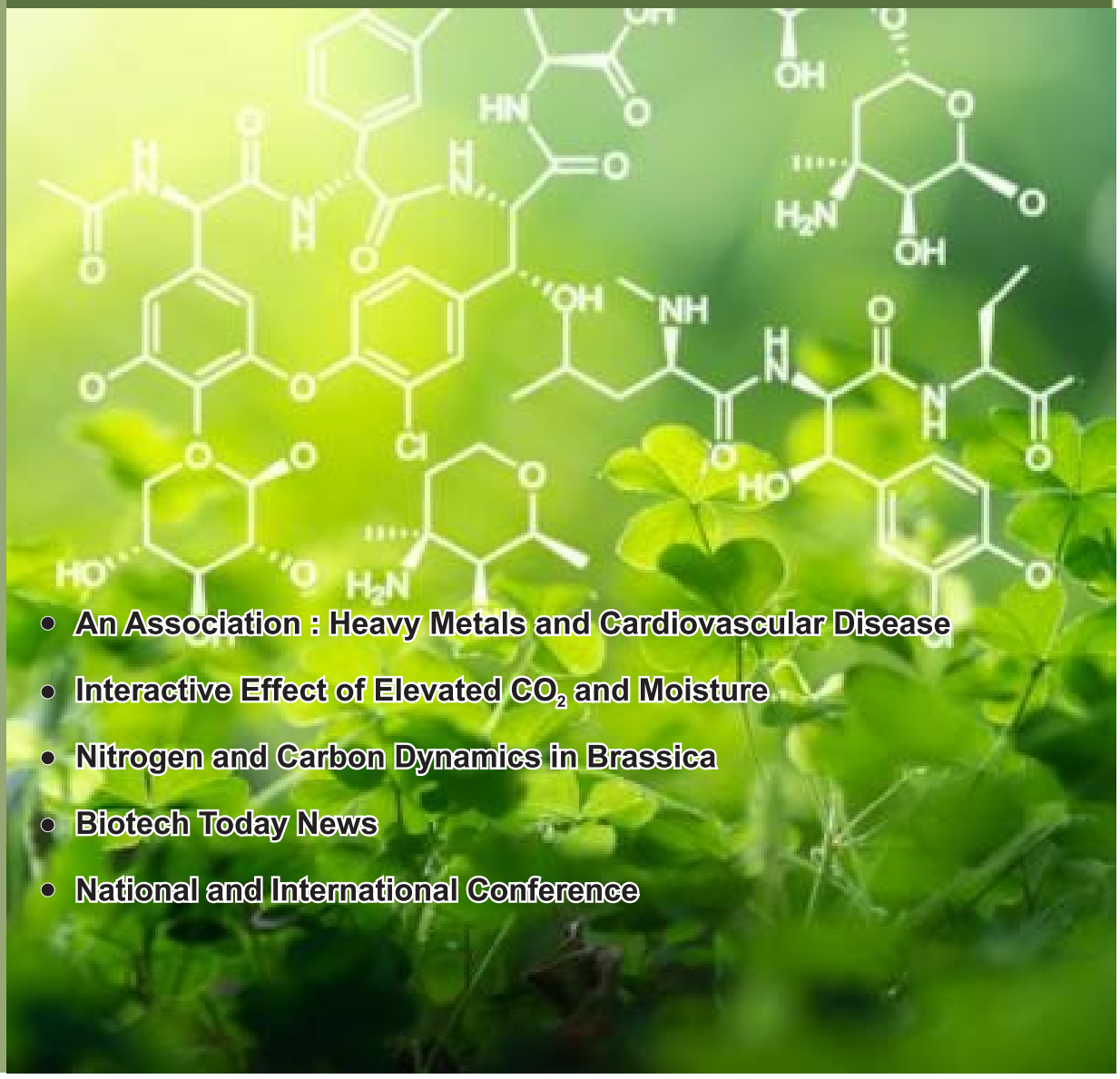

- An Association : Heavy Metals and Cardiovascular Disease
- Interactive Effect of Elevated CO_2 and Moisture
- Nitrogen and Carbon Dynamics in Brassica
- Biotech Today News
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In This Issue Page No.

REVIEW AND RESEARCH ARTICLE

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- To promote and undertake research and development and extension service in the field of agriculture and biological science.
- To explore new areas in agriculture, biological research, biotechnology crop cultivation technologies, development activity and logistics management.
- To propagate utilization on non-conventional and renewable sources in agriculture and biological research.
- To develop purely organic package for growing and cultivation of crops.
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RESEARCH ARTICLE

An Association : Heavy Metals and Cardiovascular Disease

Anshul Dhar[1*], Akriti Gaurav[2], Aditya Pathak[3], Anupam Singh[4], R. S. Sengar[5] and Pankaj Chauhan[6]

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Abstract

A growing world health concern is cardiovascular disease CVD. All deaths from CVD are not accounted for conventional risk factors. In the development of this disease, it is primarily the environmental, nutritional and lifestyle behavioural variables that are the control keys. The possible connection was less clearly established between chronic exposures to heavy metals, such as As, Pb, Cd, Hg and CVD. However, either through animal studies or by cellular and molecular research, the precise cause of CVD caused by heavy metals needs further study. In addition, to classify factors predisposing to heavy metal exposure in CVD, large-scale longitudinal research with follow-up on common people using suitable biological markers & cardiovascular endpoints may be advised. In this study, we would include a quick overview of the homeostasis of heavy metals, chapter provides a description of the evidence available for their association with CVD and the possible mechanisms involved that might justify its harmful impact. Finally, there is a review of suspected associations between biological, environmental influences.

Keywords- Cardiovascular Disease, Lead, Arsenic, Mercury, Cadmium.

Introduction

A growing issue around the globe is cardiovascular disease (CVD). Not all risk factors are well established to blame for all CVD fatalities. In the development of such a disease, it is mainly the climate, food intake and behavioural changes that serve as the control key. The potential link between the exposure of chronic high density metal like Pb, As, Hg, CD, and CVD has been less specifically established. Although the damaged process of metabolism of antioxidants & stress (oxidative) may perform a significant role.

In addition, either by in vivo testing or by cellular and molecular research, the specific process of cardiovascular disease caused by heavy metals needs further review. In addition, it could be proposed to classify the factors affecting heavy metal poisoning in CVD in prospective study with research on total populace utilizing suitable biomarkers to be used for diagnosis as well as prognosis and cardiovascular endpoints. We would provide a brief overview of homeostasis and control of heavy metals in this literature, accompanied by summary of existing data for its connection with cardiovascular disease and modes of operation by the site of harmful effects could be described. Finally, there is discussion of suspected associations between environmental & nutritional factors and genetic parameters. The pernicious effects of high density metal at

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its elevated rate of consumption is properly documented, currently concern is the probability that continuous exposure to slightly lower concentrations of heavy metals may contribute to chronic negative health consequences (Ibrahim *et al.* 2006) .Despite the significant decline in human high-density metal manifestation in current history, in many households and in many occupational environments, the risk for high consumption of these pollutants persists (WHO, 2009). Potential causes of heavy metal toxicity are beauty products such as lipsticks, Talcum powder, eye shadow, and skin lightening creams (Sainio *et al.* 2000). In classical eye makeup such as Kohl and Surma (Al-Ashban *et al.* 2004), the existence of lead has been confirmed. Henna and hair dye are documented to be very abundant in heavy metals like as lead and mercury, a common product used as Short term ink painting and dying of hair (Lekouch *et al.* 2001). Toys, some manufactured candies & spices may be other secret sources (CDC 2002; Wolf *et al.* 2005). Packaged religious Water from Zamzam, that is made accessible to pilgrims for selling, has reportedly been pulled from the marketplace for excessive arsenic containment (Alissa *et al.* 2011). Tobacco plants are especially capable of extracting cadmium from the soil and of accumulating it in the leaves (Chiba *et al.* 1992). A significant source of transmission to Cd is the smoking of cigarettes & hookah, a commonly employed smoking tool in Saudi Arabia (Saleh *et al.* 2000).

The possible relationship between exposure to chronic heavy metals and heart disease

Although the cardiovascular disease system is not usually seen as a priority for toxic metals, review papers documenting their crucial function as cardiac toxicant or elements are minimal and the primary issue of most assessments is that the antioxidant shielding processes triggering oxidative stress in the cells or DNA are disproportionate as a significant impacts of its environmental influence. It can modify genome transcription, especially dietary ingredients up regulation of gene and expression is anticipated to be liable for toxicity of high density metal. A quick description of the toxicity of heavy metals, homeostasis, will be addressed in this paper, followed by an overview of the evidence available for their link with CVD and the possible process of activity by which its poisons impacts could be explained. Finally, there is a review of suspected associations between genetic, nutritional, and environmental influences (Alissa *et al*. 2011).

Impacts of Parameters are Assessed as Possible Mediators:

These mechanisms can entail the interaction of individual variables(Sex, Age etc.); behavioural risk influences; genome influences (e.g., genetics); social influences; dose of heavy metals); health problems like diabetes, cardiovascular disease, and blood pressure); other predictive biological markers of disease like renal dysfunction, cognitive declines.

In the broadest context, the array of difficulty for cardiovascular disease variessolely from hereditary to behavioural & habitats variables. A concurrence of various factors of risk initiates CVD. The latter two have earlier shown that habitat and behavioural (comprising nutritional configuration) act an important function in most CVDs.The time of onset, dynamics and cardiovascular disease consequences, reflecting the complex pathology & physiology of cardiovascular disease are distinct for patients. The probable causes of these variations are distinct, genetically defined susceptibilities to environmental risk factors, CVD system interactions with other organs such as the immune system, and potential interactions within a person between these risk factors. The importance of environmental factors is

hardly explored, despite a growing awareness of genes, proteins, signalling pathways, and cell-cell interactions and systemic processes involved in CVD (initiation, development, and outcome) (Alissa *et al.* 2011).

Cadmium (Cd)

Cd, a widespread cyanogenetic metal, is also considered to pose serious threats to human health as an environmental and industrial waste material (WHO 1992). Cd may be a potent environmental cardiotoxic metal, and exposure would occur via pathways such as fertilizers, batteries, and water from waste matter (Menke *et al.* 2009). Cd exposure may also be caused by smoking, emissions from beverages and everyday habits (Navas-Acien *et al.* 2005). It produces toxicity following the creation of free radicals by accumulation in biological systems. Alternative research have demonstrated that Cd toxicity occurs through Necrobiosis, disruption to DNA, and aerophilous stress that can lead to cardiotoxicity and cardiovascular disease due to Cd poisoning (Prozialeck *et al.* 2006). Many researches have indicated that exposure to Cd results in several CVDs, such as coronary artery disease, infarction, and cardiovascular disease. CD increases in vitro production of reactive oxygen species (ROS) in internal organ myocytes resulting in a cyanogenetic cascade resulting in membrane degradation, while Cd intoxication increases internal organ markers (myocardial amino acid enzyme, dehydrogenase, and internal organ troponins), inhibitors (glutathione, antioxidant, enzyme and catalase) reduces catalyst activity (Saleh *et al.* 2000). A research performed in apolipoprotein E knockout (ApoE-/-) mice undoubtedly showed that CD exposure decreased total levels of steroid alcohol contributing to epithelial tissue dysfunction, plaque formation of arteriosclerosis inside the artery, increased aerophilous tension, and finally encouraged the first development of coronary artery disease within ApoE-/-mice artery (Saleh *et al.* 2000). Once exposed to CD, which causes necrobiosis in inner organ cells, aerophilous stress and pro-inflammatory mediators will increase cardiac muscle toxicity. The necrobiosis-related Bax supermolecule controls the viability of the mitochondrial membrane, while Cd causes aerophilous stress, increasing harm to the mitochondrial membrane (Chiba *et al.* 1992; Alissa *et al.* 2011; WHO, 1992). A research performed in rat hearts revealed that treatment with Cd up regulated the development of pro-inflammatory cytokines (tumour gangrene [TNF] and IL6), apoptotic stimulants (Bax and cleaved caspase-3) in the inner tissue of the organ, and altered the expression of anti-apoptotic proteins such as Bcl-2 (Chiba *et al.* 1992). In varied animal models, Cd-induced cardio toxicity should be constant due to major variations in organic phenomena, electrophysiological and contracted properties of internal organ tissue, and species heterogeneity in internal organ responses (WHO, 1992; Menke *et al.* 2009).

Since human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) provide sickness modelling and drug screening with a human-induced cell supply, several studies have stated that hPSC-CMs square measure reliable for drug-induced cardiotoxicity testing (Navas-Acien et al. 2005; Prozialeck *et al.* 2006). When management and Cd-treated hPSC-CMs were compared, major changes were found in hPSC-CM operationsIn CD-treated hPSC-CMs, researchers detected less cell viability, redoubled necrobiosis, inner organ sarcomeric disease, elevated amounts of ROS, and changed electrophysiology and internal organ arrhythmias. The distinct transcriptome profile and stimulated MAPK signal cascade and the existence of suppressed P38 MAPK in Cd-treated hPSC-CMs were revealed in the RNA sequence analysis (Menke *et al.* 2009). In the circulatory system, Cd-induced poisoning has been well demarcated.

What is missing is square measure experiments that conduct step-wise research betwixt cell lines, animal strains, and so on, to establish the easiest system model to certainly discover the effects of Cd on the activity of class vessels.

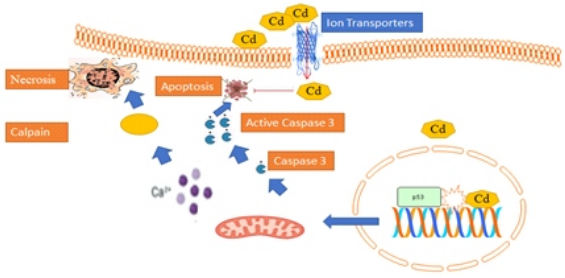

Fig. 1 Influence of Cadmium on heart.

Arsenic (As)

The growth of cardiovascular and peripheral vascular diseases has been less connected with exposure. Epidemiological research has not produced definitive evidence underlying the theory that circulatory disease is triggered by exposure (WHO, 1981). Recently, several studies have demonstrated that animal models have not been identified and accepted as viable opportunities to study As-induced circulatory diseases. A connection between (As) components and ischemic heart disease, carotid atherosclerosis, and vascular diseases has been identified in several reports that identify a higher level of As in groundwater. The exact aspects are still not fully established (Smith *et al.* 2002). In a potable water analysis that revealed a positive association with faster heart rate, relative indicators of arterial constriction have been linked to increased atherosclerosis chances (Smith et al, 2004). There is little confirmation of the Arsenic metabolism's properties on the risk of life-threatening CVD since essential toxicity has been shown by As-associated metabolites (Axelson *et al.* 1978; Chiou *et al.* 1997).

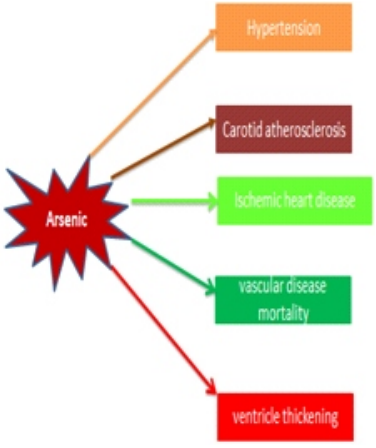

Fig. 2 Effects of arsenic on heart.

Copper

Copper (Cu), which can play a key function in the pathology of atherogenesis, is among the powerful pro-oxidant trace elements (Barceloux *et al.*1999). The crucial parameter in the control of cardiac disease pathogenesis is expected to be oxidation, since LDL cholesterol construction and generation of reactive oxygen species arise through the oxidation of such molecules (WHO, 2016). Oxygenated LDL is a pro-atherogenic factor that performs a part in the generation of atherosclerosis, and foam cell production assumes a significant part in the progression of atherosclerosis. This can contribute to the progression of (ROS) and to the accumulation of lipid molecules in the vascular wall (Moon *et al.* 2012). Scientists suggest that Copper can impair the pathogenesis of cardio vascular diseases (atherosclerosis) (Mitchell *et al.* 2011).

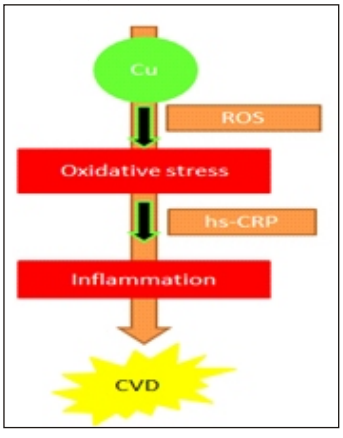

Fig. 3 Consequence of copper on heart. Cu triggers oxidative stress via imbalances in the antioxidant mechanism that raise reactive oxygen species ROS which may cause the initiation of signal transduction that stimulate inflammatory pro - inflammatory cytokines. Even so, inflammation, including cardiovascular disorders, is a characteristic of chronic diseases.

Discussion

In everyday life, the rapidly increasing global population, and the greater incidence of heavy metal exposure in industries, these criteria have contributed to the advanced consumption of elements by inhalation and food habits. Heavy metals are commonly used in industrially processed goods and could have the potential to enter living things through wastewater. Heavy metals are in significant concentrations in the environment today, but in living things they can bio accumulate. In addition, if contaminants are absorbed into the body or absorbed into the blood in multiple forms from the atmosphere, their absorption builds up in a metal-specific way in different tissues, exceeding the toxicity level after bioaccumulation. Improvement in the consideration of the toxic dose or metal content in living organisms is therefore important. In the ecosystem, the ubiquitous existence of metal delivery exhibits a possible threat to all species. Environmental contamination occurs through inhalation of toxic air, the development of plants by trace metals in infected soil, or the circulation of polluted elements into drinking water. Depending on metal features, the toxic effects of heavy metals which may have more than one transport mechanism varies since not all are eliminated when brought into the body, tissues, skin, etc. and they may be transferred in several vital organs. Heavy metals can be a cause of a large range of diseases due to these particular pathways. The toxic effects are largely due to the change in metabolic progression within the cell (DNA damage, gene mutation, formation of (ROS)) and so on. The theory of involvement in heavy metal toxicity in neurodegenerative diseases has now been generally accepted (ATDSR, 2011). Nevertheless, the harmful impact of heavy metals on the cardiovascular system are very well described. A possible correlation exists betwixt exposure to chronic metal elements and CVD. Since the death rate for this condition is very high, the process of action for heavy metal toxicity must be understood. While the disease symptoms (Atherogenesis, pro-inflammatory responses, atherosclerosis, oxidative stress, and pro-inflammatory responses) of high doses of exposure to heavy metals and their harmful consequences are well established, in examples of chronic heavy metal exposure, more research is needed to fully understand the differences in cellular processes in the cardiovascular system.

Conclusion

In present review, we addressed the possibility that high density metals cause harmful influences on the function of heart that lead in pathological and physiological changes linked to elevated development of (ROS), inflammation, Damage of DNA, cell death, and some other symptoms including abnormalities of high blood pressure. Although, the process of degradation of the circulatory system triggered by trace minerals should be investigated in more depth through. The evolution of tested in-vivo or pathological and molecular approaches planned to enhance our knowledge of metal-induced cardiovascular toxicity-related cellular mechanisms. Depending on sex and context regarding the production of CVD, different factors may be involved. Second, the degree of penetration, then the total absorption inside the body, or body load, must be differentiated. If the condition is identified, inquiries may ensure that report the detection of biological markers that correspond with the vulnerability. The chances of treating CVDs are established by enormous research on the

dose-dependent biological stages of CVD, associated with absorption of heavy metals.

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RESEARCH ARTICLE

Interactive Effect of Elevated CO_2 and Moisture Stress on Carbohydrate Partitioning Pattern

Ranjan Das*

Recieved : 20 March, **Revised** : 28 April 2021, **Accepted** : 10 May 2021, **Published** : 1 July 2021

Abstract

A study was conducted to find out the effect of elevated levels of CO_2 on two Brassica cultivars under moisture stress by comparing in the ambient condition. The experiment was carried out in Free Air CO_2 Enrichment Facility. The objective of the present study was to investigate the carbohydrate profile under such conditions. The results showed that the moisture stress significantly lowered the accumulation of various types of sugars and NSC in various stages of growth. The negative effect of moisture stress was ameliorated under higher level of CO_2 in every stages of growth and both the cultivars showed variation in response in terms of sugar, starch and NSC accumulation. The highest accumulation was recorded in RH-30 cultivar compared to Pusa Gold. The increase in accumulation non-structural carbohydrate NSC under elevated CO_2 condition and moisture stress in the later stages of growth may help in remobilization of NSC stored in the stem as reserve is important for the grain filling and development of siliquae in Brassica.

Keywords: Brassica, carbohydrate, elevated CO_2, moisture-stress and sugar,

Introduction

Emissions of green house gases resulting in global climate change affects both ecosystems and agricultural activities which ultimately damage the biodiversity, production and productivity of agriculture and food security (Allmendinger,2018, Rodrigues *et. al.*, 2016). The key variables related to climate change identified are/as rising atmospheric CO_2, increased day and night temperatures and uneven rainfall patterns which may lead to droughts and floods which have a greater impact on plant growth and development. (Ziska 2016). Carbon dioxide concentrations have risen dramatically in the post-industrial era as a result of high anthropogenic activity and are anticipated to reach 550 mol mol^{-1} in the next 50 years (Ruhil *et al.* 2015). Climate variables have a direct correlation with crop yields, and they can have a positive or negative impact on agricultural production. For example, a rise in temperature may have a detrimental impact on crop production (Das *et. al.*, 2020), potentially resulting in lower crop yields around the world (Awais *et. al.*, 2018, Lobell *et. al,.* 2011 and Rahimi-Moghaddametal, 2018). Over the past century, elevated CO_2 has been reported to increase evaporation and precipitation potential, cause greenhouse effects, and induce drought in temperate zones (IPCC, 2007) and significantly affect the anatomy , growth and yield (Das 2021a , Das 2021b and Das 2020, Das and Das 2020).

Various Scientists such as Ruiz-Vera *et. al.*, 2013; Penuelas *et al.*, 2013; Bagley *et al.*, 2015; Lavania *et al.*, 2015; Lobell *et al.*, 2014; have reported that global climate change causes an increase in atmospheric carbon dioxide concentrations, a warming climate, rising temperatures, and changing precipitation patterns, all of them have an impact on

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terrestrial ecosystem structure and function, crop productivity and water and carbon balance. In Brassica, increased CO_2 resulted in a significant increase in carbohydrate component (Uprety *et al.* (1997). Starch content increased by 37.5 %, followed by reducing (28 %), total (24%), and non-reducing sugars (22%).According to him elevated CO_2 significantly reduced protein content (10.3%) in the seeds and furthermore, CO_2 enrichment also increased the oil content by (10.4%). Because of the diluting effect, the accumulation of carbohydrates may result in a decrease in protein content (Wong 1990). According to Field *et al.* (1992), increased CO_2 alters the pattern of carbohydrate reserve accumulation in plants, as well as a significant change in plant biomass. As a result, the plant's N is diluted and the N concentrations are reduced compared to carbohydrate and this higher carbohydrate is associated with a high C: N ratio. The excess carbohydrates produced due to higher level CO_2 may serve as osmoticum and directly regulate leaf dehydration and enhance tolerance. According to Uprety and Rabha (1999), non-structural carbohydrate accumulated in Brassica as a result of elevated CO_2 aided in osmotic adjustment under moisture stress conditions. Li *et al.*, 2008 reported that the CO_2 had facilitated photosynthate assimilation, and increased photosynthate supplies from the source leaf to the sink leaf, which accelerated the growth and sink-source transition in new developing sink leaves in rice.The present investigation hypothesizes that elevated CO_2 may affect the accumulation and translocation of NSC and control the moisture deficit thereby sustaining the production. Here, we measured the sugar profile in various part of the Brassica plant under simulated condition (Free Air CO_2 Enrichment Technology) for testing the hypothesis. Our study will help to illuminate the relationships of NSC accumulation and translocation under different CO_2 levels and water regimes and will provide a scientific basis for source-sink relationship under the background of global climate change.

Materials and Methods

Plant material

Brassica cultivars viz. Brassica juncea cv. RH-30 and Brassica campestris cv. Pusa Gold were collected and grown for the present investigation.

Experimental site and growth conditions

The response of both the species to elevated CO_2 was studied using Free Air CO_2 Enrichment Technology (FACE) to simulate the doubling CO_2 concentration at, IARI, New Delhi-12. The crops were grown in the field and inside the Mid Free Air CO_2 enrichment (FACE) facility in 8 m diameter circles. An elevated CO_2 concentration of 550 µmolmol-1 was maintained throughout the crop growth period with the help of computer-based PID valves. There was no exogenous supply of CO_2 to the normal air under ambient field condition. Field was prepared by recommended agronomic practices.

Cultural practice

Farmyard manure was applied at the rate of 5 tons per hectare at the time of field preparation. The plant spacing, fertilizer application at the rate of 30 + 30:60:40 kg per hectare of nitrogen, phosphorus and potassium and other cultural practices were followed as reported by Uprety *et al.* 2001.

Moisture stress treatment

Moisture stress treatment was given by restricting irrigation and bringing the soil moisture level between 7 and 10% compared to 22–25% under irrigated condition. All the observations were taken in triplicate for each treatment at Stage-1: vegetative (25 days after sowing), Stage-2: flower bud initiation (45 DAS), Stage- 3: 50% flowering (60 DAS) and Stage-4: post flowering (75DAS).

Carbohydrates estimation

Sample extraction for estimation of sugar was done according to method reported by McCready *et al*., (1950). Fifty milliliter of aliquot of the extract was evaporated in a water bath so that the liquid did not dried out completely. Subsequently, 1ml of lead acetate was added to precipitate the colloidal substance. It was then filtered into a beaker and 3 ml of disodium hydrogen phosphate was added and made up to required volume. These aliquot was used for determining the reducing sugar by Nelson's arsenomolybdate method (Nelson, 1944). One milliliter of the aliquot was taken in test tube and made to a uniform volume of 2 ml with distilled water. It was then mixed with 1.0 ml of somogyi's copper reagent and heated in boiling water bath for 12 minutes and then cooled in running tap water. One ml of arsenomolybdate reagent was added to it and volume was made to 10 ml. Absorbance was recorded in a spectrophotometer (Model: GS570155, EC India Ltd) at a wavelength of 530 nm. A blank and two freshly prepared glucose standards were also included with each set of samples. The sugar content was calculated from a standard curve drawn from freshly prepared glucose solutions and expressed in mg g^{-1} dry weight. Five milliliter of extract was hydrolyzed by boiling with half volume of 0.5 N HCl in water bath for 30 min and neutralized at slightly acidic side with NaOH (0.5 N). The neutralized extract was made to 10 ml. This solution was used for determining the total sugar. An aliquot of this was analyzed for sugar following the same procedure as described for reducing sugar and expressed in mg g^{-1} dry weight.The reducing sugar was subtracted from the total sugar to obtain the non-reducing sugar content. Total soluble sugar (TSS) content was computed by adding reducing and non-reducing sugar content (Allen *et al*., 1988) and expressed in mg/g^{-1} dry weight.Starch content was determined by Anthrone method (Mcready *et al*., 1950). Starch content was calculated by multiplying the glucose values by 0.9 (Pucher *et al*., 1948) and expressed in mg/g^{-1} dry weight. The total non-structural carbohydrate was computed by adding TSS and starch content (Allen *et al*., 1988) and expressed in mg/g^{-1} dry weight.

Results and Discussion

Carbohydrate content in Leaves

Leaf non-reducing sugar

CO_2 enrichment brought about significant increase in the non-reducing sugar content from 18% (vegetative) to 26% (flowering) **(Fig.1 a-h)**. It was significantly greater in 'RH-30'. Moisture stress treatment significantly decreased the non-reducing sugar between 30% (vegetative), 58% (flowering). The reduction under ambient and elevated CO_2 conditions varied from 25% (vegetative) to 38% (flowering) and from 15% (vegetative) to 26% (flowering) respectively in 'Pusa Gold', whereas, between 24% (vegetative) to 35% (flowering) and 14% (vegetative) to 35% (flowering) respectively in 'RH-30'.

Leaf reducing sugar

Significant increase in the reducing sugar content of the leaves ranging from 20% (vegetative) to 26% (flowering and post flowering) was observed due to CO_2 enrichment **(Figure 1 a-h)**. Reducing sugar was significantly more in 'RH-30'. Moisture stress brought about significant decrease in the reducing sugar content varying from 36% (vegetative stage) to 56% (flowering). It varied from 29% (vegetative) to 36% (flowering) and from 17% (vegetative) to 27% (flowering) under ambient and elevated CO_2 condition respectively in 'Pusa Gold' whereas between 26% (vegetative) to 33% (flowering) 17% (vegetative) to 25% (flowering) under ambient and elevated CO_2 conditions respectively in 'RH-30'.

Leaf total sugars

CO_2 enrichment brought about significant increase in the total sugar content varying from 19% (vegetative) to 28% (flowering) throughout the growth period (Fig.1 a-h). It was significantly greater in 'RH-30'. Moisture stress significantly decreased the total sugar content between 33% (vegetative) to 57% (flowering). Reduction under ambient and elevated CO_2 condition varied from 27% (vegetative) to 37% (flowering) and from 16% (vegetative) to 20% (post flowering) respectively in 'Pusa Gold' and in case of 'RH-30' it ranged between 26% (vegetative) to 34% (flowering) and 15% (vegetative) to 18% (flowering) under ambient and elevated CO_2 condition respectively.

Carbohydrate content in stem

Stem non-reducing sugar

CO_2 enrichment brought about significant increase in non-reducing sugar content in the stem of Brassica cultivars between 16% (vegetative) to 24% (flower bud initiation and flowering) **(Fig.2 a-h)**. Non-reducing sugar content was more in the stem of 'RH-30' cultivar compared to 'Pusa Gold' throughout the growth period. Moisture stress significantly reduced the non-reducing sugar ranging from 28% (vegetative) to 48% (flower bud initiation). This reduction under ambient and elevated conditions varied from 27% (vegetative) to 34% (flower bud initiation) and from 12% (vegetative) to 21% (flower bud initiation) respectively in 'Pusa Gold', whereas, between 27% (vegetative) to 30% (flower bud initiation) and between 10% (vegetative) to 17% (flower bud initiation) in 'RH-30'.

Stem reducing sugar

CO_2 enrichment brought about significant increase in the reducing sugar content ranging from 19% (vegetative) to 25% (flowering) **(Fig.2 a-h)**. It was more in the stem of 'RH-30'. Moisture stress treatment significantly decreased the reducing sugar from 33% (vegetative) to 44% (flower bud initiation). The reduction under ambient and elevated CO_2 conditions varied from 28% (vegetative) to 32% (flower bud initiation) and from 19% (vegetative) to 21% (flowering) respectively in 'Pusa Gold', whereas, between 27% (vegetative) to 31% (flower bud initiation) and 17% (vegetative) to 20% (post flowering) respectively in 'RH-30'.

Stem total sugar

The higher CO_2 level brought about significant increase in the total sugar content of stem ranging from 17% (vegetative), 25% (flower bud initiation). Total sugar content was more in the stem of 'RH-30' **(Fig.2 a-h)**. Moisture stress treatment significantly decreased the total sugar between 30% (vegetative) to 47% (flower bud initiation). This reduction under ambient and elevated CO_2 conditions varied from 28% (vegetative) to 33% (flower bud initiation) and 15% (vegetative) to 20% (flower bud initiation) respectively in 'Pusa Gold', whereas, between 27% (vegetative) to 31% (flower bud initiation) and 13% (vegetative) to 18% (flowering) in 'RH-30' respectively.

Carbohydrate content in roots

Root non-reducing sugar

CO_2 enrichment significantly increased the non-reducing sugar content in roots from 18%, (vegetative) to 25% (flower bud initiation) throughout the growth period.Non-reducing sugar was more in the roots of 'RH-30' (Fig.3 a-h). Moisture stress treatment significantly decreased it ranging from 30% (vegetative) to 57% (flower bud initiation). This reduction under ambient and elevated CO_2 conditions varied from 24% (vegetative) to 38% (flower bud initiation) and 17% (vegetative) to 21% (flower bud initiation) respectively in 'Pusa Gold', whereas, in 'RH-30' the reduction varied between 23% (vegetative) to 36% (flower bud initiation) and 16% (vegetative) to 20% (flower bud initiation), respectively.

Root reducing sugar

CO_2 enrichment markedly increased the reducing sugar content from 20% (vegetative) to 39% (flower bud initiation) **(Fig.3 a-h)**. Reducing sugar content was more in the roots of 'RH-30'. Moisture stress significantly decreased it, which varied from 33% (Vegetative) to 48% (Flowering). Reduction under ambient and elevated CO_2 condition varied from 23% (vegetative) to 37% (flower bud initiation) and from 17% (vegetative) to 23% (flowering) respectively in 'Pusa Gold', whereas, this reduction ranged between 21% (vegetative) to 30% (post flowering) and 15% (vegetative) to 23% (flowering) respectively in 'RH-30'.

Root total sugar

Elevated CO_2 significantly increased the total sugar content in roots of Brassica cultivars from 19% (vegetative) to 31% (flower bud initiation) **(Fig.3 a-h)**. It was more in 'RH-30' cultivar. Moisture stress treatment significantly decreased it and the reduction ranged between 31% (vegetative) to 52% (flower bud initiation). This reduction under ambient and elevated conditions varied from 24% (vegetative) to 38% (flower bud initiation) from 17% (vegetative) to 27% (post flowering) respectively in 'Pusa Gold' and between 22% (vegetative) to 37% (post flowering) and 16% (vegetative) to 22% (post flowering) respectively in 'RH-30'.

Leaf starch

CO_2 enrichment significantly increased the starch content varying from 29% (vegetative) to 50% (flowering **Fig.4 a-f**). Starch content was more in 'RH-30'. Moisture stress treatment significantly decreased it varying from 36%(vegetative) to 48% (flowering). The reduction under ambient and elevated CO_2 conditions varied from 25% (vegetative) to 39% (flowering) and from 21% (vegetative) to 30% (post flowering) respectively in 'Pusa Gold' and between 28% (post flowering) to 39% (flowering) and from 20% (vegetative) to 27% (flowering) respectively in 'RH-30'.

Stem starch

Elevated CO_2 significantly increase the starch content in stem throughout the growth period.Starch was more in the stem of 'RH-30' **(Fig.4 a-f)**. Moisture stress brought about significant reduction in the starch content varying between 35% (vegetative) to 48%, (flower bud initiation). This reduction under ambient and elevated CO_2 condition varied from 33% (vegetative) to 36% (flowering) and 23% (vegetative) to 28% (flower bud initiation) respectively in 'Pusa Gold', whereas, between 26% (vegetative) to 33% (post flowering) and 20% (vegetative) to 22% (flower bud initiation, flowering, post flowering) respectively in RH-30.

Root starch

CO_2 enrichment brought about significant increase in the starch content ranging from 28% (vegetative) to 35% (flower bud initiation) **(Fig.4 a-f)**. Starch content was more in the roots of 'RH-30' cultivar. Moisture stress treatment markedly decreased the starch content ranging from 43% (vegetative), 54% (flower bud initiation). This reduction under ambient and elevated CO_2 conditions varied from 35% (vegetative) to 39% (flower bud initiation) and 23% (vegetative) to 30% (flower bud initiation) respectively in 'Pusa Gold', whereas, between 30% (vegetative) to 36% (flower bud initiation) and 21% (vegetative) to 24% (flower bud initiation) respectively in 'RH-30'.

3.5.Non-structural carbohydrate (NSC) content at different parts

Leaf

CO_2 enrichment significantly increased the non-structural carbohydrate contentin leaves of Brassica cultivars ranging from 21% (vegetative) to 26% (flowering) **(Table 1.)**. NSC was more in 'RH-30'. Moisture stress brought about significant reduction in the non-structural carbohydrate ranging between

26% (vegetative) to 45% (flowering). This reduction under ambient and elevated CO_2 varied between 31% (vegetative) to 39% (flowering) and 20% (vegetative) to 31% (flowering) respectively in 'Pusa Gold', whereas, it varied between 29% (vegetative) to 32% (flowering) and 11% (vegetative) to 25% (flowering) respectively in 'RH-30'.

Stem

Elevated CO_2 markedly increased the non-structural carbohydrate of the shoot ranging between 22% (flowering bud initiation) to 32% (post flowering) **(Table 2)**. This carbohydrates content was more in 'RH-30'. Moisture stress significantly decreased the non-structural carbohydrate ranging from 34% (vegetative) to 45% (flowering). This reduction under ambient and elevated conditions varied between 32% (vegetative) to 50% (flower bud initiation) and 22% (vegetative) to 26% (flowering) respectively in 'Pusa Gold' and between 27% (vegetative) to 32% (post flowering) and 20% (vegetative) to 22% (flowering) respectively in 'RH-30'.

Root

CO_2 enrichment significantly increased the non-structural carbohydrate in roots ranging between 28% (vegetative) to 33% (post flowering). This carbohydrate content was more in 'RH-30' **(Table 3)**. Moisture stress significantly decreased the non-structural carbohydrate content ranging between (27% flowering) to 44% (flower bud initiation). This reduction under ambient and elevated CO_2 condition varied between 33% (vegetative) to 39% (post flowering) and 17% (post flowering) to 29% (flower bud initiation) respectively in 'Pusa Gold' and 29% (vegetative) to 35% (flower bud initiation) and 20% (vegetative to flower bud initiation) respectively in 'RH-30'.

In the present investigation, larger accumulation of sugar and starch and non-structural carbohydrates appeared in leaf, stem and root in every stages of growth. These results were in conformity with findings of other workers in different crops. According to them, CO_2 significantly increased the NSC content in rice stems (Huang *et al.*, 2003; Lai *et al.*, 2015; Yoshinaga *et al.*, 2020) and and whole plant (Zhu *et al*,. 2016). For plant growth and energy metabolism, NSC plays a pivotal role as it is act as a substrate for respiration (Pan *et al.*, 2002). During reproductive development of crop especially in pre-anthesis, NSCs accumulated in stem as reserved substances are directly associated with sink strength and seed setting rate (Fu *et al.*, 2011). Ishimaru et al., 2004 and Zhao *et al.*, 2019 reported in rice that NSCs plays a significant role in improving the lodging tolerance. Between the cultivar highest accumulation of total sugar (both reducing and non reducing) and NSC was recorded in RH-30 in each stages of growth. Lower accumulation of all these parameters in post flowering stage indicated that theses photosynthates may be utilized for grain development.

The maintenance of water status in the plant by osmo-regulation due to larger accumulation of soluble sugars as well as non-structural carbohydrates in the cell-sap is a well known phenomenon. The higher amount of NSC in Brassica plant in CO_2 enriched condition may possibly help in the maintenance water status by osmo-regulation under moisture stress (Wullschleger *et al.*, 2002). This result was in conformity with the present investigation indicating NSC might be playing a defensive role during stress. Similarly, Ellsworth (1999) also reported that higher level of CO_2 might be involved in alternation of leaf dehydration tolerance in cases where CO_2 induced surplus NSC serve as osmotic for drought tolerance. Other researchers like Zhuo *et al,*. (2000) and Masle (2000) also opined that excess production of soluble carbohydrates enhanced cell division and wall expansion, however, the mechanism of these processes remains elusive. The increase in NSC in treated plant may link to higher rate of photosynthesis in Brassica Plant (Das, 2020). Ulfat *et al.*, 2021

also reported that higher level CO_2 could make better assimilation of CO_2 by enhancing antioxidant potential and make possible additional supply of photosynthates though improving activities of carbohydrate metabolic enzymes, and thereby increasingyield. The high concentration of NSC and the total mass of NSC accumulated in the stems under elevated CO_2 with level of N than those in the ambient (Cao *et al.,* 2020). From the present study it has clear that the elevated CO_2 could ameliorate drought of plants if augmented the rates of net photosynthesis which may lead to provide more substrate for osmotic adjustment during drought.

Conclusion

It is clear that accumulation of carbohydrates may possibly lead to changes in the metabolic processes of plant system which ultimately influences physiological processes like osmoregulation, source-sink relation and translocation of photosynthate in plant. Protein content of the plant may be reduced because of dilution effect, as levels of NSC and plant biomass was significantly increased under elevated CO_2 condition. So, elevated CO_2 and it's interactions with moisture stress might affect the accumulation and translocation of NSC thereby influencing yield.

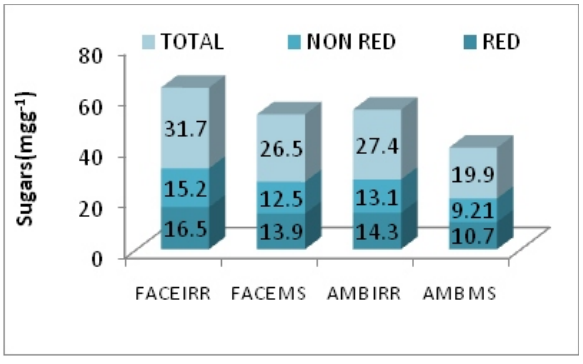

Fig. 1 (a) Interactive effect of elevated CO_2 on sugar content of leaf at vegetative stage in Pusa Gold

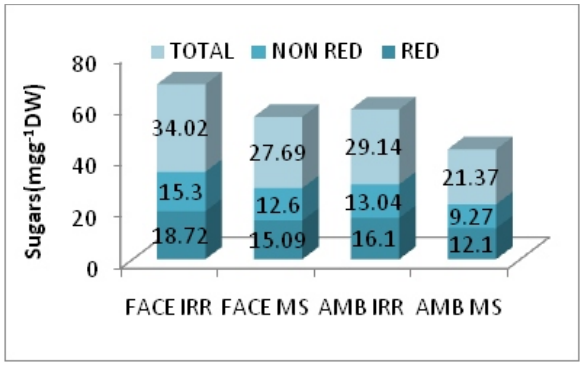

Fig. 1 (b) Interactive effect of elevated CO_2 on sugar content of leaf at vegetative stage in RH-30

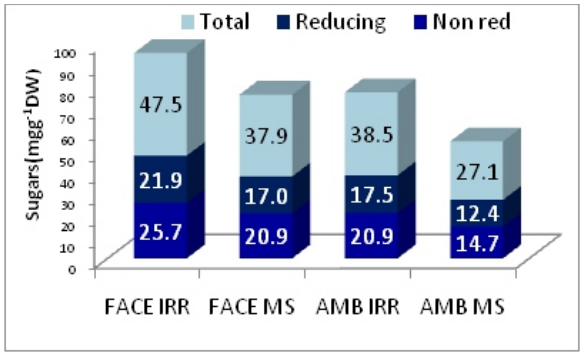

Fig. 1 (c) Interactive effect of elevated CO_2 on sugar content of leaf at Flower bud initiation stage in Pusa Gold

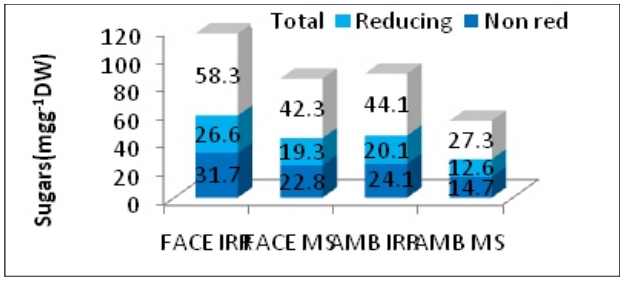

Fig. 1 (e) Interactive effect of elevated CO_2 on sugar content of leaf at Flowering stage in Pusa Gold

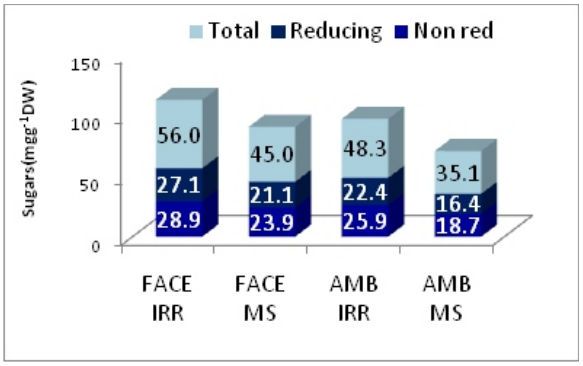

Fig. 1(d) Interactive effect of elevated CO_2 on sugar content of leaf at Flower bud initiation stage in RH-30

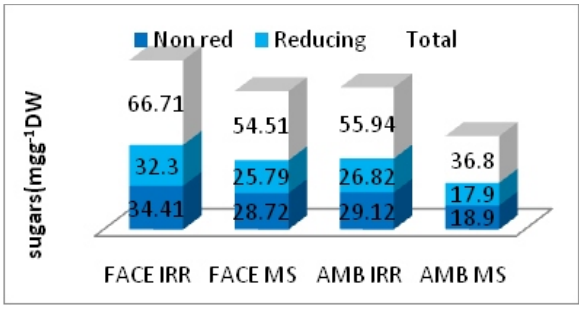

Fig. 1 (f) Interactive effect of elevated CO_2 on sugar content of leaf at Flowering stage in RH-30

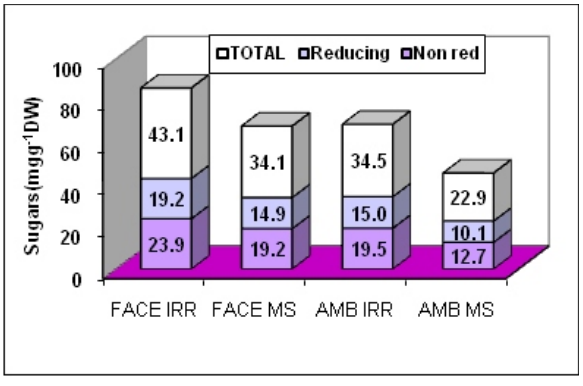

Fig. 1 (g) Interactive effect of elevated CO_2 on sugar content

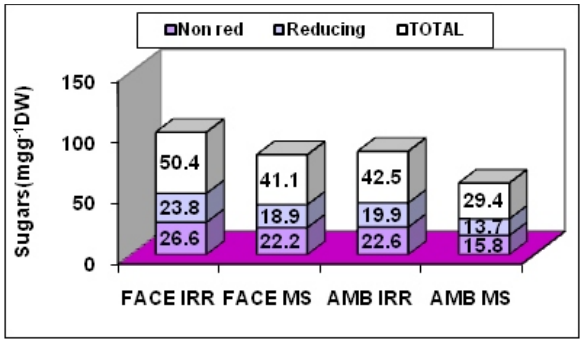

Fig. 1 (h) Interactive effect of elevated CO_2 on sugar content of leaf at post Flowering stage in Pusa Gold

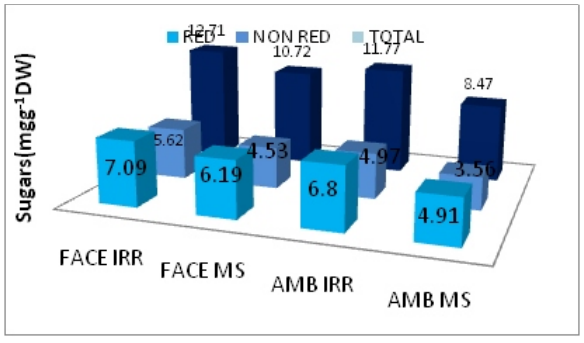

Fig. 2 (a) Interactive effect of elevated CO_2 on sugar content of stem at vegetative stage in Pusa Gold

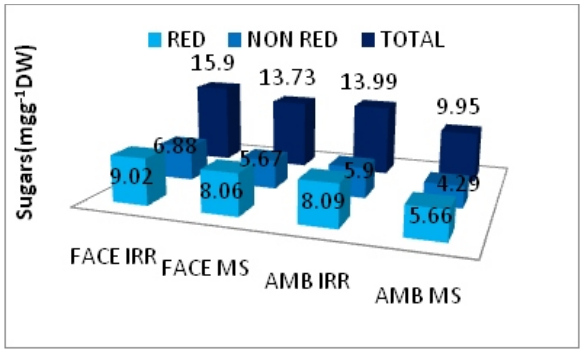

Fig.2 (b) Interactive effect of elevated CO_2 on sugar content of stem at vegetative stage in RH-30

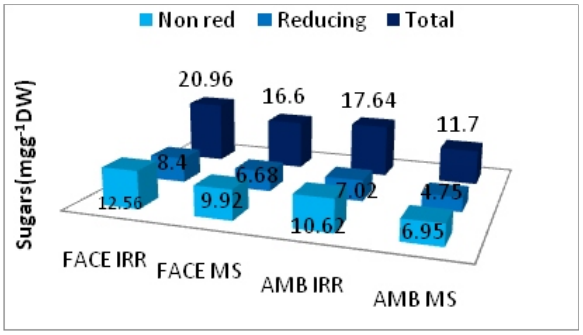

Fig. 2 (c) Interactive effect of elevated CO_2 on sugar content of stem at flower bud initiation stage in Pusa Gold

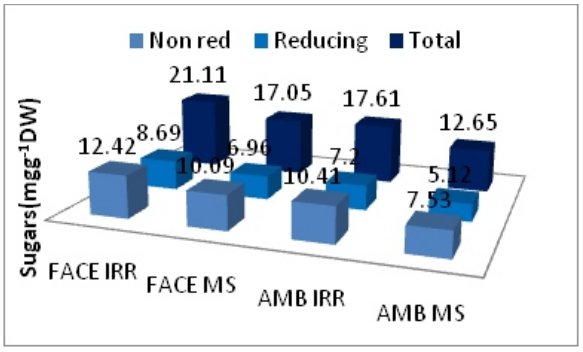

Fig.2 (f) Interactive effect of elevated CO_2 on sugar content of stem at flowering stage in RH-30

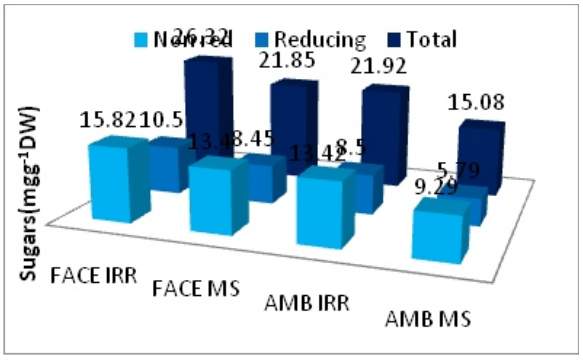

Fig.2 (d) Interactive effect of elevated CO_2 on sugar content of stem at flower bud initiation stage in RH-30

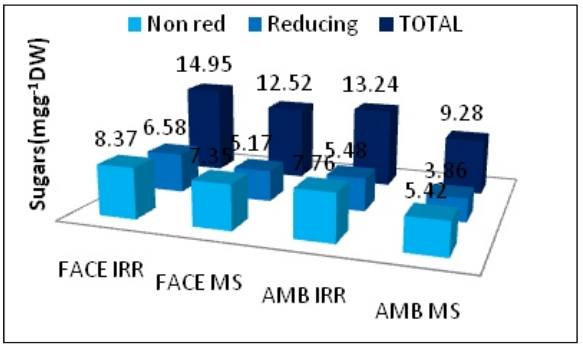

Fig. 2 (g) Interactive effect of elevated CO_2 on sugar content of stem at post flowering stage in Pusa Gold

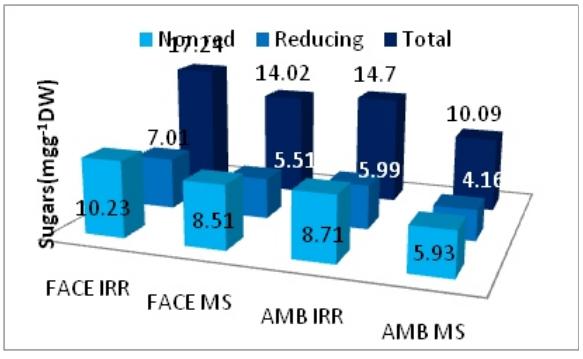

Fig. 2 (e) Interactive effect of elevated CO_2 on sugar content of stem at flowering stage in Pusa Gold

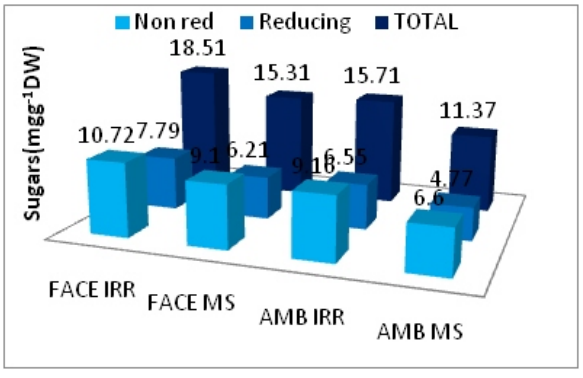

Fig. 2 (h) Interactive effect of elevated CO_2 on sugar content of stem at post flowering stage in RH-30

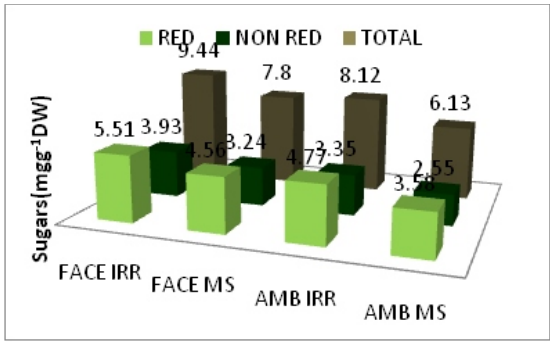

Fig. 3 (a) Interactive effect of elevated CO_2 on sugar content of root at vegetative stage in Pusa Gold

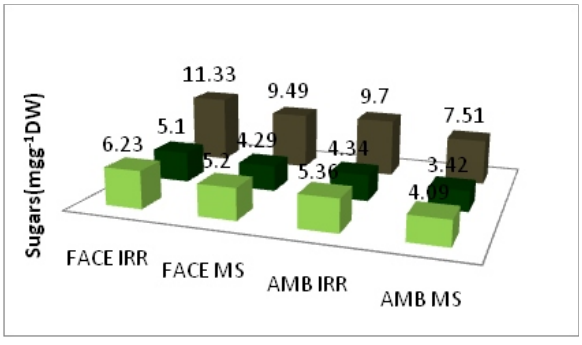

Fig. 3 (b) Interactive effect of elevated CO_2 on sugar content of root at vegetative stage in RH-30

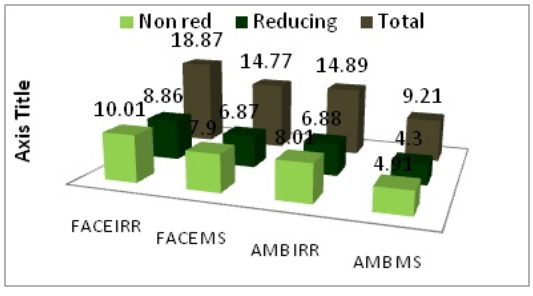

Fig. 3 (c) Interactive effect of elevated CO_2 on sugar content of root at flower bud initiation stage in Pusa

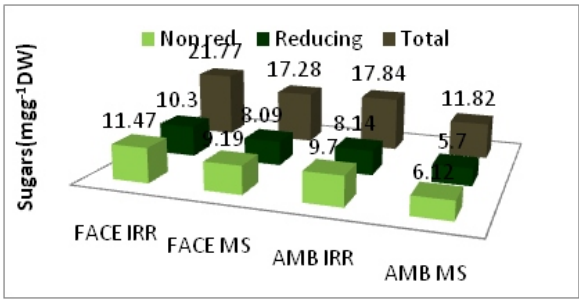

Fig. 3 (d) Interactive effect of elevated CO_2 on sugar content of root at flower bud initiation stage in RH-30

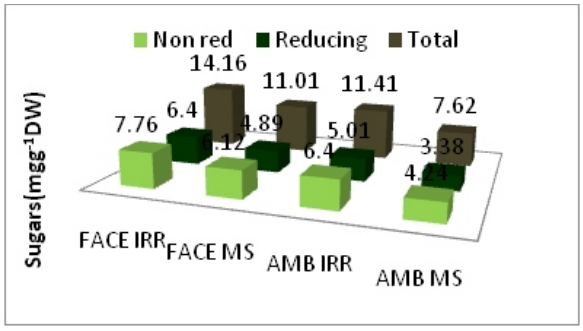

Fig. 3 (e) Interactive effect of elevated CO_2 on sugar content of root at flowering stage in Pusa Gold

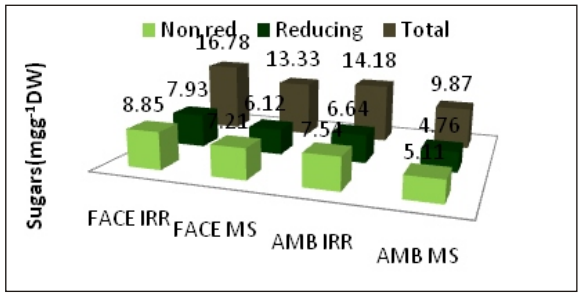

Fig. 3 (f) Interactive effect of elevated CO_2 on sugar content of root at flowering stage in RH-30

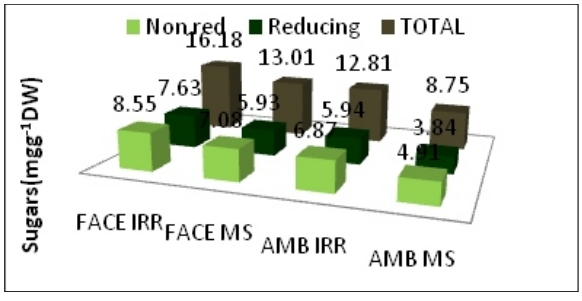

Fig. 3 (g) Interactive effect of elevated CO_2 on sugar content of lroot at post flowering stage in Pusa Gold

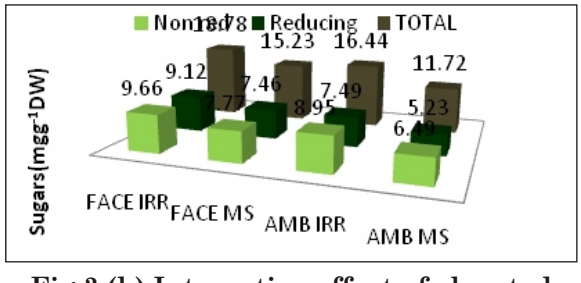

Fig 3 (h) Interactive effect of elevated CO_2 on sugar content of root at post flowering stage in RH-30

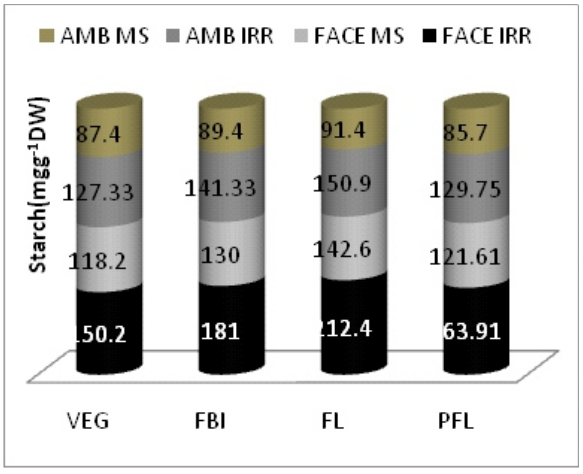

Fig. 4 (a) Interactive effect of elevated CO_2 on starch content of leaf at various stages of growth in Pusa Gold

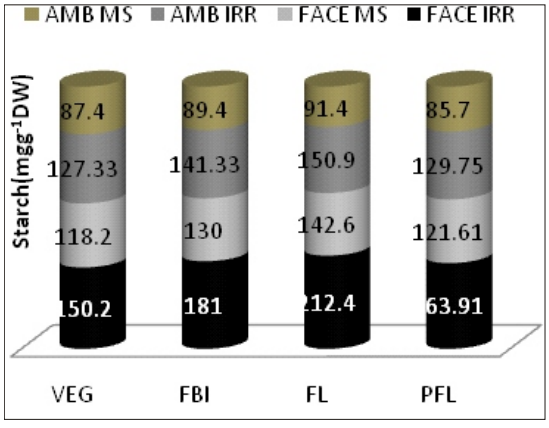

Fig. 4(b) Interactive effect of elevated CO_2on starch content of leaf at various stages of growth in RH-30

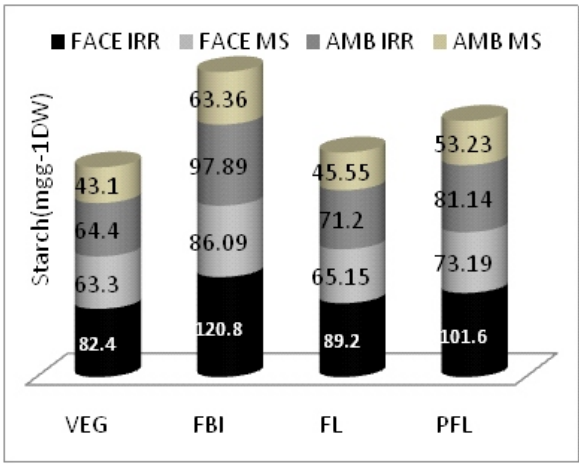

Fig. 4 (c) Interactive effect of elevated CO_2 on starch content of stem at various stages of growth in Pusa Gold

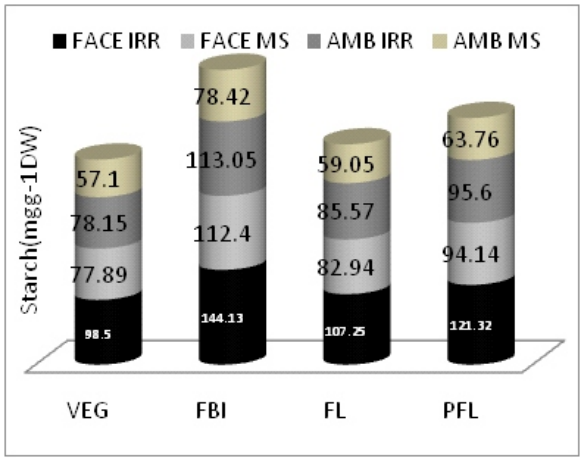

Fig. 4(d) Interactive effect of elevated CO_2 on starch content of stem at various stages of growth in RH-30

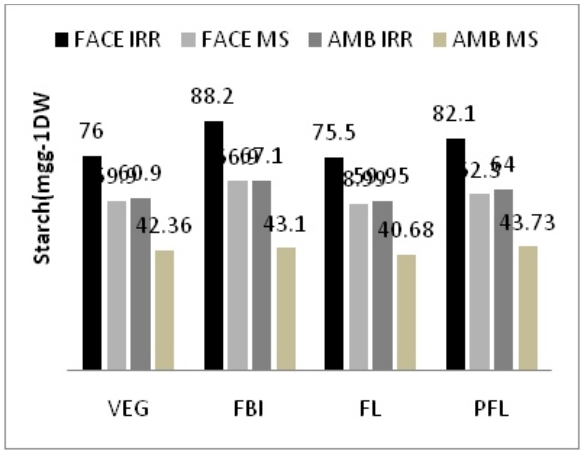

Fig. 4(f) Interactive effect of elevated CO_2 on starch content of root at various stages of growth in RH-30

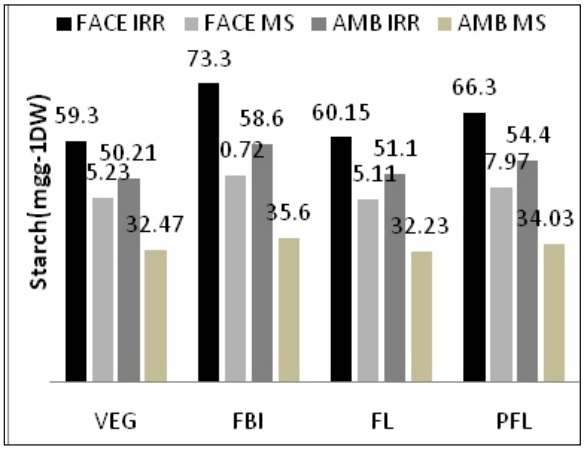

Fig. 4 (e) Interactive effect of elevated CO_2 on starch content of root at various stages of growth in Pusa Gold

FACE = Free air CO_2 enrichment, IRR= Irrigated, MS= Moisture stress, AMB= Ambient

Table 1. Interactive effect of elevated CO_2 and moisture stress on Non structural carbohydrate (NSC) on leaves of Brassica species at various stages of growth

Treatments	Vegetative	Pre flowering	Flowering	Post flowering				
	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30
FACE IRR	181.76	181.08	228.82	279.37	270.72	332.96	207.05	247.06
FACE MS	144.70	161.29	170.32	217.51	184.87	246.91	155.62	194.99
AMB IRR	155.25	173.04	176.17	220.40	194.96	249.34	164.24	196.99
AMB MS	107.33	122.47	116.54	149.52	118.73	169.30	108.69	139.99
Var.	13.33	23.67	44.78	21.45				
CO_2	16.34	19.44	22.67	16.77				
Var. x CO_2	23.31	25.21	32.06	21.47				
MS	14.63	13.56	27.54	15.44				
Var. x MS	21.344	19.81	30.94	19.89				
CO_2 x MS	23.33	22.24	38.94	24.77				
Var. x CO_2 x MS	30.56	27.12	55.07	30.22				

FACE = Free air CO_2 enrichment, IRR= Irrigated, MS= Moisture stress, AMB= Ambient

Table 2. Interactive effect of elevated CO_2 and moisture stress on Non structural carbohydrate (NSC) on stem of Brassica species at various stages of growth

Treatments	Vegetative	Pre flowering	Flowering	Post flowering				
	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30
FACE IRR	95.11	114.40	141.76	170.45	106.44	128.36	116.55	139.83
FACE MS	74.02	91.26	109.99	134.25	79.17	100.00	85.72	109.45
AMB IRR	76.12	92.14	151.53	134.97	85.90	103.19	94.39	111.31
AMB MS	51.57	67.05	75.06	93.50	55.64	71.70	62.51	75.13
Var.	12.83	10.44	15.21	9.68				
CO_2	4.82	3.78	6.21	4.40				
Var. x CO_2	6.82	5.89	8.64	6.22				
MS	8.35	7.44	7.76	7.78				
Var. x MS	11.81	10.21	9.44	11.01				
CO_2 x MS	13.45	12.62	11.35	12.23				
Var. x CO_2 x MS	16.70	15.12	14.56	15.64				

Table 3. Interactive effect of elevated CO_2 and moisture stress on Non structural carbohydrate (NSC) on root of Brassicaspecies at various stages of growth

Treatments	Vegetative	Pre flowering	Flowering	Post flowering				
	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30
FACE IRR	68.74	87.33	92.17	109.97	74.31	92.28	82.48	96.88
FACE MS	53.01	69.39	65.19	84.18	56.13	72.32	67.65	79.99
AMB IRR	58.33	70.60	73.49	84.94	62.52	74.13	67.23	80.44
AMB MS	38.60	49.87	44.81	54.92	39.86	50.55	42.79	55.45
Var.	7.12	8.56	6.12	6.89				
CO_2	3.34	4.01	3.23	3.43				
faautoVar. x CO_2	5.56	6.12	5.34	5.42				
MS	4.76	5.04	4.12	4.32				
Var. x MS	7.66	8.02	7.04	7.23				
CO_2 x MS	9.44	10.23	8.75	9.24				
Var. x CO_2 x MS	12.32	13.77	11.12	11.98				

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RESEARCH ARTICLE

Phytochemical analysis and mineral composition of Safed and Pili Shatavar (*Asparagus recemosus*) and Assessment of its Bioactivity

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Abstract

The aim of present study was to investigate phytochemical and mineral composition of pili shatavar and safed shatavar and its bioactivity. *A. racemosus* is a biochemical factory of different class of metabolites including saponins, sterols terpenoids, alkaloids, tannins, glycosides, flavonoids, coumarine, carbohydrates and amino acid. The minerals composition was analysed by AAS. Ca was observed to be highest in pili shatavar 5.276 µg/g. The Mn content was highest in safed shatavar (0.934 µg/g). The iron content observed to be 1.134µg/g in pili satavar and1.172µg/g in safed shatavar. The Zn content was highest in pili shatavar (0.364µg/g). Cu content was trace amount in pili and safed shatavar respectively. The TPC was maximum in pili shatavar (69±0.012 mg/gm) as compared to safed shatavar (36.5±0.33 mg/g). The TFC was maximum in pili shatavar (15.32±0.29 mg/gm) and minimum in safed shatavar (10.09±0.12mg/g). Root extract from pili shatavar showed highest total antioxidant activity (IC_{50}=189.77±4.36) as compared to safed shatavar (IC_{50}=254.57±4.25). However root extract of safed shatavar (IC_{50}=199.19±0.38) observed to have a strong *In-vitro* anti-inflammatory effect relative to the pili shatavar. The health promoting phytochemical of *Asparagus racemosus* and their antioxidative potential could be utilized in pharmaceutical industries.

Key words : *Asparagus racemosus*, phytochemicals, mineral composition, Anti-inflammatory, Antioxidants

Introduction

The term medicinal plant known to a diversity of plants that have medicinal values. These plants are abundant source of components that can be used for drug synthesis (Khalid *et al*., 2012). The parts of medicinal plants that are used are different types of seeds, root, leaf, skin, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have indirect or direct therapeutic effects and are used as medicinal agents. The medicinal effects of plants are due to secondary metabolite production of the plants. Keeping this in consideration there have been increased waves, of interest in the field of the research in natural substances chemistry. *Asparagus racemosus* is an powerful medicinal plant of the subtropical and tropical region commonly known as Shatavar, Satavar or Satmuli (Parihar and Sharma, 2021) Shatmuli etc. which belongs to family Asparagaceae and considered as a rasayana, a herbal medicine that promotes common health by rising cellular ability in the Ayurveda (Dahanukar, 2000). The presence of flavonoids, considered a good sequestrant of free radicals, indicates that this plant could have antioxidant properties.

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Saponins are related to antibacterial activity and glycosides reported for lowering blood pressure (Shao, 1997; Oketch-Rabah, 1998). This plant is ideal for all-weather cultivation in Uttarakhand and will become a source of income for many young people (Joshi, 2016). The chemical composition of methanolic extracts from pili and safed shatavar (*Asparagus racemosus*) was analyzed through GC/GCMS (Leema *et al.*, 2020). Both the plant type exhibited the presence of several bioactive compounds. Therefore, the present study focused on investigation of phytochemical and mineral composition of the rhizmatous extracts from pili shatavar and safed shatavar and biological activity of the methanolic extracts. The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane and Botting, 1995).

Materials and Methods

Rhizomes of pili shatavar and safed shatavar were collected from M.R.D.C. Pantnagar, Uttarakhand, to study the phytochemical analysis.

Preparation of extracts

The shade dried rhizomes were coarsely powdered and subjected to successive extraction, using soxhlet apparatus.

Phytochemical analysis

Phytoconstituent (carbohydrate, protein, tannins, flavonoids, phenols, alkaloids, steroids, saponins, terpenoids and cardiac glycosides) screening of safed and pili shatavar was performed through standardized methods proposed by earlier (Tripathi 2015; Nandagoapalan *et al.*, 2016).

Estimation of total Phenol

The phenolic assay of both the extracts of *A. racemosus* was calculated quantitatively by the method of Singleton and Rossi (1999). Phenol reacts with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and result in the formation of blue coloured complex, the molybdenum blue, which is measured at 765 nm.

Estimation of total flavonoids content

TFC was determined by the method of Kim *et al.*, (2003). The solution was mixed well and absorbance of colour developed was taken at 510 nm.

Mineral analysis by atomic absorption spectroscopy (AAS)

Copper, Zinc, Manganese, calcium and Iron content measurement

An atomic absorption spectrophotometer (AAS) under the present investigation was used to measure iron, zinc, copper, calcium and manganese described by Kirkbright *et al.*, (1966).

The standard belong to the element was feeded along with DDW to Atomic Absorption Spectrophotometer to standardize the instrument to read concentration in the samples having the given element within the standard range.

Bioactivity of safed and pili satawar

Determination of total antioxidant activity

Total antioxidants content was estimated by the method of Prieto *et al.*, (1999). The assay was based on the reduction of Mo (VI) to Mo (V) by the extracts and subsequent formation of green phosphate/Mo5 complex. Absorbance of the sample was measured at 695 nm.

In-vitro anti-inflammatory, activity

In-vitro anti-inflammatory, activity was determined by standard method described earlier (Kar *et al.*, 2012) with slight modifications. The sample consisted of 2 mL of extracts at different concentrations ranges from (50, 100, 150, 200, and 250µg/mL) with 200µL of fresh albumin protein. 2.8mL of phosphate buffered saline (pH 6.4) is added to the reaction mixture to make up 5mL.

Double distilled water was taken as control. Then the prepared reaction mixtures were kept in a BOD incubator (±37° C) for 15min and heated at 70° C for 5min. After cooling the reaction mixture, by keeping that at room temperature for a while their absorbance was, measured at 660nm. Diclofenac sodium at same concentration was taken as positive control (standard) and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula given below-

% inhibition =100 × (Vt / Vc - 1)

Where, Vt = Absorbance of test sample

Vc = Absorbance of control

Results and Discussion

Rhizomes of pili shatavar and safed shatavar were collected from M.R.D.C. Pantnagar to study the phytoconstituents and biological activities of extracts.

Phytochemical analysis

The qualitative phytochemical profiling of different extracts obtained in the methanol reveals that the rhizomes of A.racemosus is a biochemical factory of different class of metabolites which include sterols, saponins, terpenoids, alkaloids, tannins, glycosides, carbohydrates, flavonoids, coumarine and amino acid and Protein (Table 1). It has been reported earlier that tannin, phenols and steroids can be useful in the treatment of inflamed and ulcerated tissue and may show anticancer activity. Saponin, flavanoids and glycoside reported to produce inhibitory effect on inflammation. Flavanoids have been repoted to maintained membrane permiability (Malla *et al.*, 2013). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities, Alkaloids are versatile heterocyclic nitrogen compounds with reported antimicrobial activity against fungal or bacterial phytopathogens (Kappers *et al.*, 2005).

Table 1: Qualitative phytochemical analysis of methanolic extracts of safed and pili shatavar

S. No.	Phytochemical analysis	SSME	PSME
1.	Sterols	+	+
2.	Saponins	+	+
3.	Terpenoids	+	+
4.	Alkaloids	+	+
5.	Tannins	+	+
6.	Glycosides	+	+
7.	Carbohydrates	+	+
8.	Flavonoids	+	+
9.	Coumarine	+	+
10.	Amino acid	+	+

SSME=safed shatavar methanolic extract, PSME=pili shatavar methanolic extract. +indicates the presence of constituents Several bioactive phytoconstituents are possessed by medicinal plants that can be applied in treating cancer, inflammation, and can be used as antioxidative and antimicrobial agents because they are safe and less harmful as compared to the synthetic ones. In the present study, total phenol, flavonoids were examined in *Asparagus racemosus* (pili shatavar methanolic extracts) PSME and (safed shatavar methanolic extracts) SSME.

Phenols, being omnipresent in plants, are low molecular weight secondary metabolites with a hydroxyl group (-OH) bonded with an aromatic hydrocarbon used as antioxidants, anti-carcinogenic, anti-mutagenic, metal ion chelating agents, etc. (Alam *et al.*, 2013). Due to their responsive actions against pro-oxidants/oxidants, their total content in plants becomes an important parameter in assessing the antioxidant potential. In the present study, the total phenol content in both varieties of *Asparagus racemosus* was determined by using the Folin-ciocalteu reagent method. The results were then interpreted by providing a direct comparison of sample's activity with standard antioxidant, gallic acid. The TPC (mg GAE/g extract) was maximum in PSME (69±0.012mg/gm GAE) as compared to SSME (36.5±0.33 mg/g GAE) Table 2.

Table 2: Total phenol content (mg/gmGAE) in roots extracts of Asparagus raecemosus (Mean±S.D.).

Phytochemical assay	PSME	SSME
Total phenolic content (TPC)	69±0.012 mg/gm GAE	36.5±0.33 mg/g GAE
Total flavonoids content (TFC)	15.32±0.29 mg/gm of CNE	10.09±0.12 mg/g CNE

PSME= pili shatavar methanolic extract, SSME=safed shatavar methanolic extract

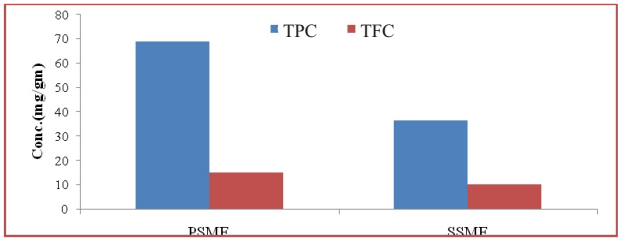

Fig 1: TPC and TFC of Pili and Safed Shatavar

Flavonoids as secondary metabolites are responsible for multiple colouration of plant parts and antioxidant activity. Upon consuming in diet, they reduce the jeopardy of several ailments like melanin abnormalities, cancer, Alzheimer's disease, diabetes, etc. (Patel *et al.*, 2018). Considering their significance, in the present study, total flavonoid content (TFC) in both varieties of *Asparagus racemosus* was determined using the aluminium chloride ($AlCl_3$) colorimetric method. The experimental data is interpreted by providing a direct comparison of sample's activity with standard antioxidant compounds like catechin equivalent (CNE). In the present study, the total flavonoid content is expressed as mg catechin equivalent (CNE)/g. The TFC (mg CNE/g extract) was maximum in PSME (15.32±0.29 mg/gm of CNE) and minimum in SSME (10.09±0.12mg/g CNE).

Quantitative determination of total phenols and total flavanoids were also reported by (Tripathi *et al.*,2015). The studies showed 586 mg/100g of total phenolics and total flavonoids in the roots of the *A. racemosus* was (0.56 mg/gm).

Mineral analysis by atomic absorbance spectroscopy (AAS)

In the current study the calcium(Ca) was found highest in pili shatavar (5.276µg/g) as compared to safed shatavar (3.41µg/g). Calcium is very important in muscle contraction, oocyte activation, building strong bones and teeth, blood clotting and fluid balance within cells. The requirements of Ca are greatest during the period of growth and during pregnancy, when breast feeding (Pravina *et al.*, 2015).

The Mangnese (Mn) content highest in safed shatavar (0.934µg/g) and lowest in pili satavar (0.78ug/g). Manganese ions function as cofactors for a large variety of enzymes Mn particularly essential in detoxification of superoxide free radicals in organism that must deals with elemental oxygen (Roth *et al.*, 2013).The Iron (Fe) content was recorded in pili shatavar (1.134µg/g) and in safed shatavar (1.172µg/g). It has been been reported that iron is an essential trace metal and show various biochemical role in the body, including binding of oxygen in hemoglobin and acting as a main catalytic center in several enzymes (Iversen 2007).

The zinc (Zn) content was recorded lowest in safed shatavar (0.364µg/g) and highest in pili shatavar (0.444µg/g). In cellular membrane structure and function zinc plays important role. It acts as a potent antioxidant as well as necessary for growth and development of healthy body tissues, regulation of insulin and proper immune system, (Shen, 2008). Cu content was the trace amount (0.102µg/g) and (0.042µg/g) in pili and safed shatavar respectively. Copper is an important part of numerous enzymes participating in the synthesis and degradation of protein, carbohydrates, lipid and nucleic acid as well as in the metabolism of other micronutrients (Krupanidhi *et al.*, 2008) (Table 3).

Table 3: Mineral analysis of *Asparagus racemosus*

S. No.	Minerals	Safed shatavar(ug/g of dry wt.)	Pili shatavar(ug/g of dry wt.)
1.	Calcium	3.41	5.276
2.	Manganese	0.78	0.934
3.	Copper	0.042	0.102
4.	Iron	1.172	1.134
5.	Zinc	0.364	0.444

The present study on micronutrients in *Asparagus racemosus* is in agreement with the previous study (Mohanta *et al.,* 2003). Trace minerals present in roots of *Asparagus racemosus* such as zinc, manganese, copper, cobalt along with calcium, magnesium, potassium zinc and selenium .This plant also contains important vitamins and minerals such as A, B1, B2, C, E, Mg, P, Ca, Fe and folic acid (Mishra *et al.*, 2013).

Total antioxidant activity

The total antioxidant activities increase significantly with the concentration of each extracts. In the present study roots extracts from PSME shows highest total antioxidant activity (PSME; IC_{50}=189.77±4.36ug/ml) with respect to ascorbic acid used as standard having the value of IC_{50}=153.69±0.09ug/ml. The variation of total antioxidant activity increases with the concentration of the extracts. The data obtained by performing the total antioxidant activity revealed that the extracts from roots of SSME exhibited good total antioxidant activity IC_{50}=254.57±4.25 ug/ml as compared to the ascorbic acid (Table 4, Fig. 2).

The TAA values in both extracts were significantly different in each variety at different concentrations. (Hossain *et al.*, 2012) have been evaluated the total antioxidant activity of roots of *Asparagus racemosus*. Ethanolic extracts of *A. racemosus* was found to contain highest total antioxidant activity (639.925±64.78.88 mg/gm) ascorbic acid equivalent. Methanol extracts of *A. racemosus* was found to posses the total antioxidant activity (616.92±53.88 mg/gm ascorbic acid equivalent).

Table: 4 IC_{50} values of various extracts of A.racemosus

S.N.	Sample Name	IC_{50} values (μg/mL) in triplicate			Mean IC_{50} values with standard deviation (μg/mL)
		1st	2nd	3rd	
1.	PSME	193.74	185.10	190.46	189.77±4.36b
2.	SSME	251.14	253.25	259.33	254.57±4.25c
3.	Ascorbic Acid	153.73	153.74	153.59	153.69±0.09a

PSME= pili shatavar methanolic extract, SSME=safed shatavar methanolic extract. Data analysed with one way analysis found to be significant at p<0.050, values within a column followed by single letter (a, b , c, d, e) show significant varietal difference by duncan's test.

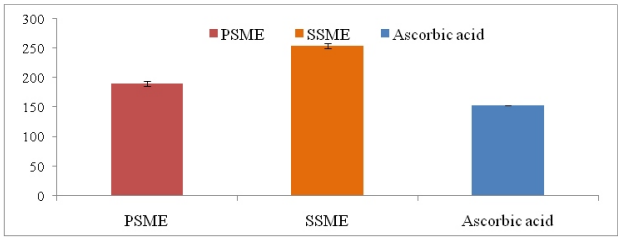

Fig 2 IC_{50} values of various extracts of *A. racemosus*

In-vitro anti-inflammatory activity

Anti-denaturation of albumin protein method was used to evaluate the *In-vitro* anti-inflammatory property of the extracts of *A. racemosus*. The results summarized in Table 5. Both the extracts protected the albumin against heat induced denaturation. The percentage of albumin protection against heat increased with increasing concentration. The present findings showed a concentration dependent inhibition of protein (albumin) denaturation by extracts from 50ppm to 250ppm. Sodium diclofenac at the same concentration ranges from 50ppm to 250 ppm was used as the reference drug and it also exhibited a concentration dependent inhibition of protein denaturation.

Extracts obtained from the safed shatavar methanolic extracts (SSME, IB_{50}= 199.19±0.38)

shows a strong anti-inflammatory effect relative to the diclofenac sodium (IB_{50}= 22.88±0.40). The pili shatavar methanolic extract, showed good anti-inflammatory property with IB_{50} value of 215.03±0.14 as compared to the safed shatavar (Table 5, Fig.3).

The clinical treatment of inflammatory diseases is dependent on drugs which belong either to the non-steroidal or steroidal chemical therapeutics. The nonsteroidal anti-inflammatory drugs such as aspirin, indomethacin, and ibuprofen inhibit early steps in the biosynthesis pathway of prostaglandins by inhibition of COX enzymes and are the main drugs used to reduce the untoward consequences of inflammation (Albert *et al.*, 2002). However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use. The use of steroidal drugs as anti-inflammatory agents is also becoming highly controversial due to their multiple side effects (Vanden Worm *et al.*, 2001).

Therefore, a demand has arisen for the development of newer anti-inflammatory agents from natural sources with more potent activity and with minor side effects as substitutes for chemical therapeutics. There are some Indian medicinal plants with their active principles having anti-inflammatory activity are reported by Chatterjee and Pal (1984). The results of the present preliminary study stated that extracts obtained from A.racemosus possessed discernible *In-vitro* anti-inflammatory effect against the denaturation of albumin protein. Further definitive studies are necessary to find out the mechanisms and constituents responsible for its anti-inflammatory actions.

Table:5 IB_{50} values (ppm) of extracts of *A. racemosus*

S. N.	Sample Name	IB_{50} values (ppm) in triplicate			Mean IC_{50} values with SD (ppm)
		1st	2nd	3rd	
1.	PSME	215.12	214.88	215.10	215.03±0.14c
2.	SSME	199.62	199.06	198.89	199.19±0.38b
3.	Diclofenac sodium	22.74	22.12	22.88	22.58±0.40a

PSME= pili shatavar methanolic extract, SSME=safed shatavar methanolic extract. Data analysed with one way analysis found to be significant at ($p<0.050$), values within a column followed by single letter (a, b , c, d, e) show significant varietal difference by duncan's test.

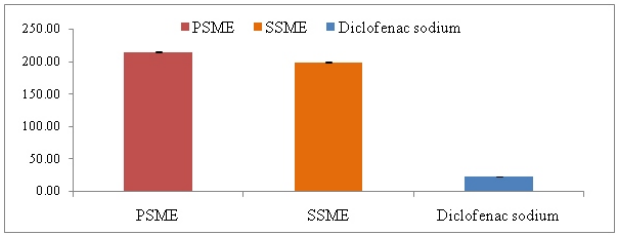

Fig.3 IB_{50} Values or roots of *A. racemosus*

Anti-inflammatory effect of alchoholic extracts of *A. racemosus* rhizomes on Sprague Dawley rats. It has been reported that *A. racemosus* roots extracts showed significant anti-inflammatory activity against, Carrageenan-induced pedal inflammation in rats (Battu *et al.,* 2010).

Conclusion

The study suggests that potential antioxidant activity of extract of Asparagus racemosus rhizomes indicates its protective role against oxidative damage and as an important natural antioxidant that has the potential to control inflammation. The extract due to its antioxidant activity could be utilized in pharmaceutical industries and food sector. Further research in this direction will be utilized for strengthening its real potential in various sectors.

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RESEARCH ARTICLE

Bio-Active Compounds and its Immunomodulatory Properties

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Abstract

Bioactive compounds are secondary metabolites produced by plants.The bioactive compounds like Curcumin, Resveratrol, Quercetin, â-Glucan, Lycopene, Genistein and others isolated from various plants are found to be act very effectively as Immunomodulators. The immunomodulators produce variety of immune responses by stimulating or supressing immune responses. Along with immunomodulatory properties, these bioactive compounds have many other pharmacological properties like anti-inflammatory, Anti-mutagenic, anti-carcinogenic, cardio and neuroprotective and many others. The immunostimulants stimulate the immune responses of immune system when any pathogen enters human body as in case of bacterial and viral infections. These are generally administered when body's immunity is decreased due to various reasons like aging, health conditions etc., Imunosuppresants supress immune responses of immune system. These are generally administered during organ transplantation to suppress the immune responses. Immunomodulators not only modulate immune responses but also used in treatment of various types of diseases like Malaria, Cancer, Alzeimer's and to improve functioning of immune system etc., The present article highlights the immunomodulatory effects of Curcumin, Resveratrol, Quercetin, â-Glucan, Lycopene and Genistein.

Keywords: Bioactive compounds, diseases, immunomodulators, pharmacology

Introduction

Immunomodulators are any compounds that have the capability of altering the immune responses. These are two types, immunostimulants and immunosuppresants. Immunostimulants stimulates or activates the immune system and immunosuppresants supresses or inactivates the immune system. Immunomodulatory compounds are bioactive compounds present in plants. Human body has three different types of immunity namely Innate immunity, Active immunity and Passive immunity.

Immunomodulators function in the following ways (Nazneen)

- They reduce inflammation and prevent nerve damage that might cause multiple sclerosis symptoms by inhibiting the immune system from attacking nerves in the brain and spinal chord.
- They aid in the slowing or halting of cancer cell proliferation.
- They inhibit the function of interleukin, a chemical that causes inflammation in the body.
- Bioactive compound is any substance that has biological activity related to its ability to modulate one or more metabolic processes which results in better health conditions (Luisa, Matteo and Amalia, 2015). These are usually the secondary metabolites of plants which include lycopene, resveratrol, lignan,

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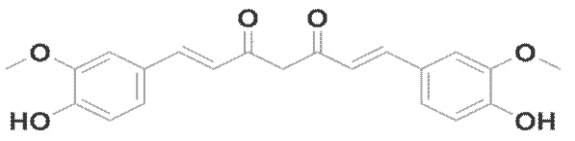
tannins, indoles etc. The bioactive compounds produced by plants, act as anti-inflammatory compounds, anticancer agents, antioxidants, analgesics, antiseptics, angioregulatory, immunomodulatory and have many other functions (Anderson). There are many immunomodulating compounds like Curcumin, Resveratrol, Epigallocatechol-3- gallate, Quercetin, Colchicine, Capsaicin, Andrographolide, Genistein, â-glucan, Flavopiridol, Compretastatin, Lycopene etc., Among the many immunomodulatory compounds, only few compounds like Curcumin, Resveratrol, Quercetin, â-Glucan, Lycopene, Genisteinwhich are more effective are discussed in the present article (Arulmozhi *et al.*, 2019).

Curcumin

Curcumin is a phytopolyphenol found in the dried root of *Curcuma longa* L., a ginger rhizome belonging to the Zingiberaceae family was first isolated by Vogel and Pelletier, in 1815. (Kunnumakkara *et al.*, 2017). The main secondary metabolites of *Curcuma longa* L. are demethoxycurcumin and bis-demethoxycurcumin (Maria and Gunawan, 2014). *Curcumin derived* from the pulverised rhizome of Curcuma longa L. is used as a food spice (Prasad and Aggarwal, 2011), while Curcumin derived from turmeric root is utilised as a colouring agent (Bradford, 2013). It is made up of a combination of resin and turmeric oil.

Chemical characteristics

The IUPAC designation for Curcumin is 1,7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3,5-dione given by Lampe, Milobedzka, and Kostaneski , in 1910. It's a Beta-Ketone, which is methane with two hydrogen atoms replaced by feruloyl groups that looks like orange-yellow needles.



(1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione

Pharmacological properties

Curcumin contains antiseptic, antioxidant, anti-inflammatory (Jakubczyketal *et al.*, 2020), analgesic, anticarcinogenic (Tomeh, *et al.,* 2019), antiviral, antibacterial (Zheng *et al.*, 2020), antimutagenic, cardioprotective, and neuroprotective activities. Curcumin has the ability to modulate gene expression- both by destroying cancer cells and by promoting healthy cell function (Javeri and Chand, 2016). It is also associated with improved cognitive abilities and lowered pervasiveness of dementia (Paliwaletal *et al.*,2021).

Mechanism of action

Curcumin acts as epigenetic modulator. It regulates epigenetic alterations via inhibiting DNA methyltransferases (DNMT's); histone modifications by histone acetyltransferases and histone deacetylases (HDAC's) and microRNA's (miRNA). It acts as a DNA binding agent and interacts with transcription factors. These mechanisms are interwined and play an important role in the progression of tumerogenesis. It is a potential source to reverse epigenetic modifications and efficiently regulate the expression of genes and molecular targets that are involved in promotion of tumerogenesis (Hassan *et al.*, 2019).

Curcumin as Immunomodulator

In 2011, Minche, Taramelli and Vivas, studied the innate immune response of Curcumin in Malarial infection. They found Curcumin dramatically boosted the macrophage phagocytic activity in mice and improved non-inflammatory latex bead phagocytosis in murine macrophages *in-vitro*.

It has elevated CD36 surface expression on human monocytes/macrophages and CD36-dependent phagocytosis. However, it inhibited pro-inflammatory cytokine responses and adhesion molecule expression in human endothelial cells *in-vitro*.

On the other hand, Ranjitha (2018) reported that Curcumin inhibits tumour cell transformation, proliferation, and metastasis at several stages of the cell cycle. It works against cancer through regulating transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes.

Side effects

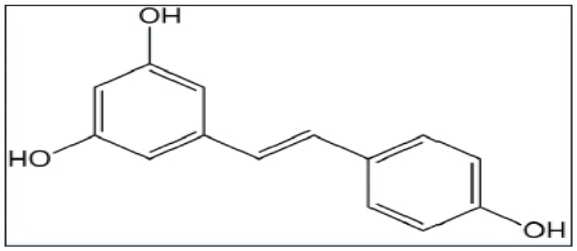
Nausea and diarrhoea are possible side effects of high doses and long-term use of curcumin (Shehzad *et al.*, 2010). Curcumin has a low oral bioavailability due to its hydrophobic nature. When it was orally administered, it undergoes conjugation, and results in the formation of Curcumin glucuronide and sulphates in the intestinal and liver walls. However, piperine improves the bioavailability of curcumin by inhibiting glucoronidation in the liver and intestine (Takahashi *et al*., 2009).

Resveratrol

Resveratrol is a natural polyphenol present in over 70 plant species, including grapes, cranberries, mulberries, pistachios, and peanuts, as well as foods such as wine, grape juice, and berries. It's a non-flavonoid found in darkly coloured fruits and vegetables (Krstonosiae *et al.,* 2021). It was discovered in the root of *Veratrum grandiflorum* O. Loes for the first time by Takaoka, in 1939.

Chemical Characteristics

Resveratrol's IUPAC designation is 5-[(E)-2-(4-Hydroxyphenyl)ethen-1-yl]benzen e-1,3-diol given by Takaoka, in 1940. Other names are Trans-3,5,4'-Trihydroxystilbene and trans-Resveratrol. It appears as a white powder with a tinge of yellow.



Pharmacological properties

Resveratrol has long been thought to have anti-aging (Baxter, 2008), antioxidant (Joanna, Aleksandra and Mieczyslaw.,2017), anti-inflammatory (de SA Coutinho *et al.*,2018), antiproliferative, angio-regulatory (Billack *et al.,* 2008), autophagy enhancing properties (Das and Maulik., 2006) and Cancer Chemo preventive properties (Jang *et al.*, 1997).

Mechanism of action:

The mechanism of action for resveratrol is controversial. (Park *et al*.,2012)have shown that resveratrol inhibits cAMP – dependent phosphodiesterases, directly generating a chain of events that converge in the activation of the energy -sensing metabolic regulators, SIRT1, AMPK and PGC -1á.

Resveratrol as Immunomodulator

Chen *et al*., (2019) created Resveratrol Dry Suspension (RDS) with a final composition of 2.93 percent Resveratrol. They found RDS is unaffected by extremes of temperature, humidity, or light. When administered into the body of an immunocompromised mouse, it enhanced the spleen index and promoted the proliferation of splenic lymphocytes. In chronic inflammation, it also suppressed inflammatory reactions, capillary permeability, auricular swelling response, and granuloma formation.

Resveratrol's antioxidant and anti-inflammatory characteristics protect against diseases such as cancer, diabetes, and Alzheimer's.

The anti-inflammatory property is also utilised as a treatment for Arthritis. However, Salehi *et al.*, (2018) stated that Resveratrol's antioxidant and anticancer properties suppress all phases of carcinogenesis.

Side effects

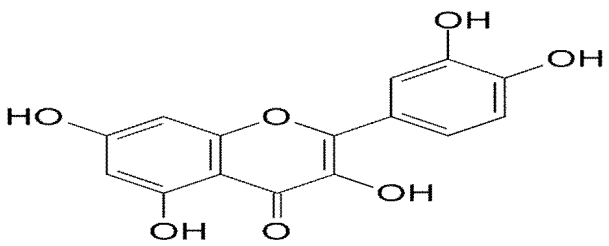
Resveratrol has low oral bioactivity because of its high lipophilicity (Abdullah *et al.,* 2021). Greater doses of resveratrol may slow blood coagulation and increase the risk of bleeding in patients suffering with bleeding disorders.

Quercetin

Highest levels of quercetin were found in Onions, Apples, Honey, Red Grapes, Cherries, Citrus Fruits, and Green Leafy Vegetables by Albert in 1937. It is a pigment that adds colour to many fruits and vegetables. It is a bioflavonoid that is frequently utilised. Onion skin and outer rings have more quantity of Quercetin (Arabbi *et al*., 2004; Lombard *et al*., 2005; Kwak *et al*., 2017). Apple peels contain a large amount of it.

Chemical characterization

The IUPAC designation for Quercetin is 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychro men-4-one given by Albert in 1937. Another name for quercetin is Sophoretin. It is a pentahydroxyflavone with five hydroxy groups at positions 3, 3', 4', 5, and 7 and has a yellow hue to it.



Pharmacological properties

Quercetin offers potential therapeutic use in the prevention and treatment of a variety of disorders, including cardiovascular disease, cancer, and neurological disease (Muhammet *et al*., 2016). It has neurotoxic as well as neuroprotective properties (Batiha *et al*., 2020). It is also used to treat heart and blood vessel disorders, high blood pressure, prostate infections, allergies, and upper respiratory infections (Annie, 2021).

Mechanism of action

Quercetin promotes neurogenesis and neuronal lifespan via regulating a variety of kinase signalling pathways including AKT/PKB tyrosine kinase, P13 -kinase and protein kinase C (PKC). It is also known for its potential to improve memory and cognitive deterioration during ageing.It is having neuroprotective impact and potentially involved in the prevention of neurological disorders (Suganthy *et al*., 2016).

Quercetin as Immunomodulator

Quercetin has been shown to exert antioxidant, anti-inflammatory and anticancer activities in a number of cellular and animal models, as well as in humans through modulating the signalling pathways and gene expression involved in these processes (Muhammet *et al*., 2016). Quercetin-3-O-á-L rhamnopyranoside showed immunomodulatory effects against Influenza A virus (Batiha *et al*., 2020). Quercetin with different dosages of (500,1000,1500 mg/day) in combination with azathioprine (100 mg/day) produced an immunemodulatory action through the reduction of interleukin-6, intercellular adhesion molecule-1 and complement proteins while elevating the serum level of interleukin-10 (Chitra *et al*., 2001). It showed immunemodulatory effect on dysregulated Th1/Th2 cytokine balance in mice with both type 1 diabetes and allergic asthma (Ravi Kumar and Kavitha, 2020). It also possessed anti-fatigue effects by prolonging retention times, decreasing levels of blood lactate. It could also improve immune function of fatigue by decreasing tumor necrosis factor- levels and elevated interleukin -10 levels (Zhang, 2017).

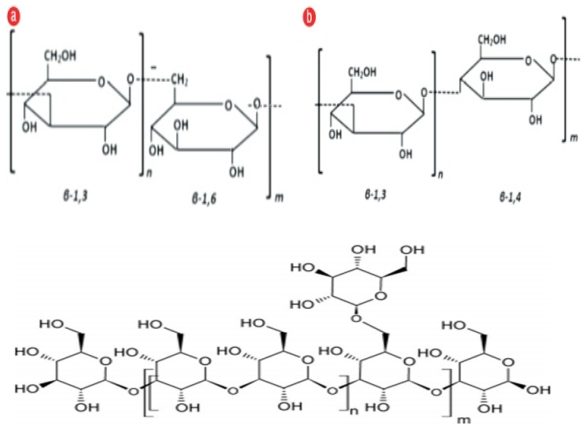
Side effects

According to early research, a Quercetin by-product can cause protein function loss (Boots and Haenen, 2007). In high dosages it might harm the kidneys. As a result, certain antibiotics may be less effective. It may raise the risk of bleeding by enhancing the effects of Warfarin (Coumadin), Clopidogrel, and Aspirin medicines. It may induce headaches or tingling in the arms and legs after being treated with Quercetin (Annie, 2021).

-glucan

-glucan is a polysaccharide. The sources of this compound varies based on different characteristics like molecular weight, solubility, glycosidic linkages and degree of branching. Traditionally the major source of beta–glucan is cereals. It is found in abundance in cell walls of yeast, bacteria, fungi and in endospermic and aleuronic walls of the cereal grains like barley, oats and millets (Novak and Vetvicka, 2008). It is also found in seaweeds like laminarian species (Laroche and Michaud, 2007).

Chemical characteristics



The molecular formula of -glucan is $C_{18}H_{32}O_{16}$ and made up of linear glucose chains with (1-> 3) and (1->4) linkages in endosperm cell walls of cereals.The -glucan found in yeast and fungi was made up of (1->3) linkages and (1->6) linkages (Du *et al.*, 2019).

Pharmacological properties

-glucans are well known biologic response modifiers that act as immunostimulants against infectious illnesses, cancer (Brown and Gordon, 2003; Borchers *et al.*,1999).p Anotheractivity of -glucan discovered in the mid 1980's was its potential to stimulate hematopoiesis in a manner similar to that of granulocyte monocyte colony stimulating factor (GMCF) (Liu *et al.*, 2009). It was shown to defend against infection with bacteria and protozoa in numerous experimental models (Trine *et al.*, 2009). It was proven to improve antibiotic efficacy in infections caused by microorganisms that are resistant to antibiotics. It was found to be effective in reversing them yelo -suppression caused by chemotherapy medications (Wagnerova *et al.*, 1993).

Mechanism of action

Michael *et al.*, (1991) have studied â-glucans for their hypocholesterolemic effects which include reducing cholesterol and bile acid absorption in the intestine by binding to glucans,shifting the liver from cholesterol syntheses to bile acid production and fermentation of short chain fattyacids by intestinal bacteria (Sao *et al.*, 2013).

– glucan as Immunomodulator:

– glucan has long been recognized to be an activator of cellular immunity (Helen *et al.*, 2009). Macrophages are activatedwhen â-glucan binds to a specific receptor (most notably CR3 and Dectin 1).This activation is made up of a number of interconnected mechanisms such as enhanced chemokinesis and chemotaxis. -glucan is also involved in fight against cancer (Godfrey *et al.*, 2009). The antitumor and antibody treatment effectiveness is significantly improved by immunotherapy with â-glucan, as a result -glucan collaboration is a very important factor for a potential immunotherapy combination.

Side effects

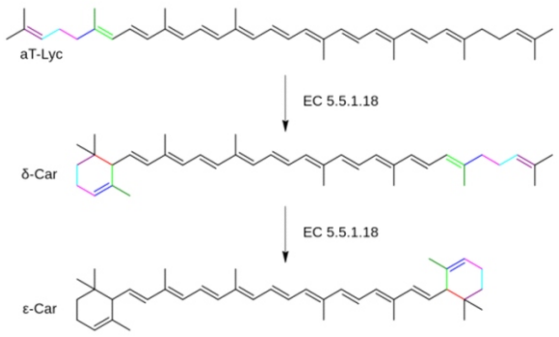
–glucan is mostly safe. However, some of the side effects include diarrhea, nausea, vomiting, dizziness, joint pain and rashes (Steimbach *et al.*, 2021; Pan *et al.*, 2019; Chan *et al.*, 2009).

Lycopene

Lycopene is a fat soluble red carotenoid pigment found mostly in tomatoes and other red fruits like papaya, pink guava,water melon, red oranges and rosehips etc., (Camara , 2013).

Chemical characteristics

Lycopene is a linear unsaturated hydrocarbon carotenoid with molecular formula $C_{40}H_{56}$ having molecular weight 536.9 g/mol.The IUPAC designation for lycopene is (6E,8E,10E,12E,14E,16E,18E,20E,22E,24E,26E)-2,6,10,14,19,23,27,31-octamethyldotriaconta-2,6,8,10,12,14,16,18,20,22,24,26,30-tridecaene.



Pharmacological properties

Lycopene has anti oxidative, anti-proliferative, anti-inflammatory properties (Heber *et al.*, 2002). Lycopene lowers prostate cancer, regulates cell cycle progression,cell proliferation and impacts the insulin like growth factor intracellular pathway by inhibiting androgen receptor expression (Carmen Avendano *et al.*, 2015). Moselhy and Almslmani (2008) have demonstrated the antioxidant defense with strong chemopreventive effect of lycopene and melatonin against DMBA-induced Breast cancers to reduce lipid peroxidation and considerably boost the activities of superoxide dismutase (SOD), glutathione peroxidase andcatalase (CAT) (Matchado *et al.*, 2018).

Mechanism of action

Lycopene is a powerful anti-oxidant. It can trap singlet oxygen and minimize mutagenesis in the Ames test. It inhibits human cancer cell development by interfering with growth factor receptor signaling and cell cycle progression specifically in prostate cancer cells at physiological doses with no indication of toxicity or cell apoptosis. Based on human and animal cells studies Connexin 43 gene was identified that is increased by lycopene and facilitates direct intercellular gap junctional communication (GJC) (Heber and lu, 2002).

Lycopene as Immunomodulator

Lycopene at a concentration of 2-12 mol/L has been shown to suppress blood platelet aggregation induced by arachidonic acid,collagen and adenosine diphosphate with an efficacy comparable to that of aspirin. It has impact on the transcription factor stimulating cell proliferation (PDGF- platelet derived growth factor) and it may also limit smooth muscle cell growth (Ezzat *et al.*,2020). It has also been shown to boost quinacrine activity and block Wnt -TCF signaling in cancer cells via adenomatous polyposis coli, while having no effect on MCF-10A (a non-tumorigenic epithelial cell lining) in normal breast cells (Preet *et al.*, 2013; and Verma *et al.*, 2021).

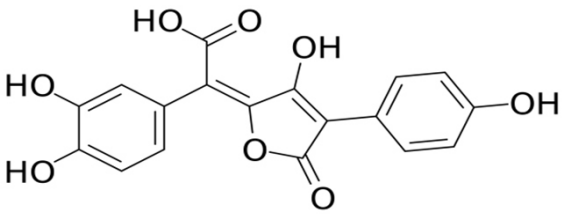
Side effects

Lycopene is safe to eat when eaten in foods. Excessive lycopene consumption can cause lycopenemia, a skin disorder characterized by an orange or red discoloration.The disorder is innocuous and can be cured by consuming a low-lycopene diet (Snijesh and Singh, 2014 anbd Wong, 2021).

Genistein is an isoflavanoid. It was first discovered in the leguminous plant *Genistia tinctoria* L. It is also found in cauliflower, sunflower,broccoli and barley(Javad Sharifi -Rad *et al*., 2021).

Chemical characteristics

The IUPAC designation for Genistein is 5,7 -Dihydroxy -3-(4-hydroxyphenyl)-4H-1benzopyran -4-one. Its molecular formula is $C_{15}H_{10}O_5$ and molecular weight 270.24 g/mol.



Pharmacological properties

Genistein acts as an anticancer agent that inhibits leukotriene production, persuades apoptosis, inhibits platelet aggregation, decreases bioavailability of sex hormones, induces differentiation in cancer cells and inhibits leukotriene production (Fotsis *et al*, 1995; Boik 1996; Peterson *et al* ,1995; Kennedy ,1995 and Matsukawa *et al*.,1993).

Mechanism of action

Goh *et al*., (2022) have focused on the mechanisms of genistein's anti-inflammatory action that includes NF- inhibition, PG's inhibition,inhibition of Ino's and inhibition of ROS. Regulation of ER's and suppression of protein tyrosine kinases, nuclear factor â (NF-) and topoisomerases I and II are the key mechanisms of genistein's effect.

Genistein as immunomodulator

Ghaemi *et al.*, (2012) reported that ingestion of genistein dramatically boosted lymphocyte proliferation and LDH release. Genistein therapy resulted in a considerable increase in -interferon. Furthermore, when compared to the control group,the therapy had a substantial therapeutic impact in tumour models. The effect of genistein on tumour growth can be linked to its effect on cytolytic-activity,lymphocyte proliferation and -interferon production.

Side effects

Genistein have adverse effects on developing female reproductive tract, gastro intestinal upset, allergic reactions, loss of appetite,constipation and ankles swelling (Christiansen, 2021).

Conclusions

The bioactive compounds like Curcumin, Resveratrol, Quercetin, â-glucan, Lycopene and Genisteinwere isolated and characterized from various plant sources and found to have Immunomodulator properties. Due to this property and others, these compounds are used in treatment of many diseases. Curcumin is used in the treatment of Malaria, Alzheimers's, rheumatoid arthritis, inflammatory bowel disease and common malignancies like colon,stomach, lung, breast and skin cancers; Resveratrol in the prevention and treatment of certain types of Cancer, heart disease, high cholesterol, inflammation, blood clotting, Hay fever and weight loss; Quercetin in atherosclerosis, high cholesterol, heart disease, diabetes, cataracts, Hay fever, peptic ulcer, Schizophrenia, inflammation, asthma, gout, chronic fatigue syndrome, chronic infections of prostate and cancer; -glucan in diabetes, hypercholesterolemia, obesity, cardiovascular diseases, neurodegenerative diseases, eczema and cancer; Lycopene in different types of disorders such as Alzheimer's, Parkinson's, cerebral ischemia, epilepsy, Huntington's disease, depression and diabetes and Genistein in cardiovascular, menopause symptoms and osteoporosis. Hence, many more bioactive compounds from plants to be isolated, characterized and tested for their biological activities which can be used in therapeutics.

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Conflict of Interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Recent Advancements and treatments in Recurrent Spontaneous Abortion : An Overview

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Abstract

Two or more successive pregnancy losses before the 20th week of pregnancy are referred to as recurrent spontaneous abortion (RSA). Recurrent spontaneous abortion has an etiology that is mostly unknown, multifactorial, with a lot of discussion about diagnosis and care. In this review we talked about the causes and the method to treat recurrent spontaneous abortion (RSA). Anatomical, gastrointestinal, and placental disorder, hormone concerns, illness, cigarettes and alcohol consumption environmental conditions, mental illnesses and traumatic life experiences, as well as some coagulation and protein deficiency responsible for immune system control, are all logical etiologic triggers.

In addition, the immune response plays an important role in human reproduction. Infertility, polycystic ovary syndrome, IVF success, obstetrical success, and male gonadal function have all been linked to vitamin-D. Low vitamin-D levels can boost the risk of obstetric problems during pregnancy. The majority of (RSA) cases, however, are undescribed, which can be attributed to autoimmune and isoimmune antibodies, which could play a role in pregnancy immunologic failure and abortion. The ability to recognize pregnancy immunologically is critical for the continuation of pregnancy. Anti-HLA antibodies may be prevented by inadequate identification of antigens from an unborn child or increased HLA exchange with the parent. Abortion is linked to lower levels of antiidiotypic antibodies (Ab2), anti-paternal cytotoxic antibodies (APCA), and mixed lymphocyte reaction blocking antibodies (MLR-Bf) in women with (RSA), according to several reports.

Thrombolytic therapies such as aspirin as well as heparin, intravenous immunoglobulin (IVIg) treatment, lymphocyte vaccination with parental lymphocytes, and newly used (RSA) drugs are all efficient.

Keywords: Recurrent Spontaneous Abortion, Immunologic Factors, Natural Killer Cell, Aspirin, Heparin.

Introduction

In terms of its social and economic consequences, spontaneous miscarriage is a significant problem. Females are delaying conception until they are in their thirties or forties more often these days, and fertility is declining as a result, the rate of spontaneous abortion rises after the age of 30–35 years. Abortion is characterized as the termination of a pregnancy before 20 weeks of pregnancy or when the baby weighs less than 500 gm; it can be spontaneous, threatened, imminent, complete, or incomplete (Lin *et al.,* 2018). The most common pregnancy complication is abortion (Moradinazar *et al.* 2020).

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In around half of RSA cases, no etiologic element has been reported (Yang *et al.* 2017). Unsafe abortion is still a significant public health issue around the world, with the World Health Organization reporting 21.6 million unsafe abortions in 2008 (Lin *et al.* 2018). For all pregnant women, a spontaneous miscarriage is a devastating loss. It affects around 1% of all females (Li *et al.* 2017).

Recurrent spontaneous abortion (RSA) was historically described as the 3 or more consecutive miscarriage just before 20th week of gestation (Pandey *et al.* 2005; Sarno *et al.* 2019). However, since clinical investigation and possible interventions might be too late if started after the third miscarriage, this term should be redefined to reflect the new social behavior of pregnancy at an older age. The American Society for Reproductive Medicine updated its concept of RSM in 2008, stating that it now includes two or more aborted pregnancies (Wang *et al.* 2017; Sarno *et al.,* 2019). Infertile couples have a higher risk of spontaneous miscarriage than the general population, according to an early analysis, and patients with a history of RSM have a higher incidence of infertility. However, another study found that the risk of spontaneous abortion is not higher in pregnancies conceived with assisted reproductive technology (ART) than in pregnancies conceived naturally. Recurrent spontaneous abortion has an underlying cause that is sometimes uncertain and can be highly variable, with considerable discussion about assessment and treatment. Recurrent miscarriage can be caused by a number of factors like, chromosomal, infection, smoking, low vitamin D levels (Li *et al* 2019; Sereshki *et al* 2014; Tian *et al.,* 2020; Samimi *et al.,* 2017). and alcohol intake, as well as genetic, anatomical, endocrinological, stress factors and placental abnormalities (Adib-Rad *et al.*, 2019). In addition, certain autoimmune and isoimmune factors can play a role in RSA-positive women's pregnancy immunologic collapse (Wu *et al.*, 2017).

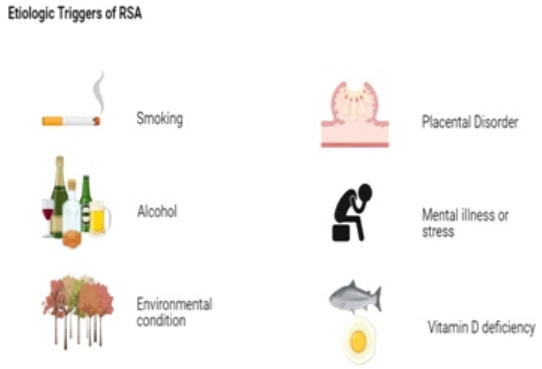

Fig. 1 Etiologic Triggers of RSA

Changes in HLA-G molecule expression, T-helper-1 cytokine sequence, and natural killer (NK) cell cytotoxicity may also induce abortion in women with RSA. The immunological interaction between the mother and the embryo is a 2 way interaction defined on the one side by perinatal antigen exposure and on the other side by the mother's immune system's recognition and reaction to these antigens. Antithrombotic therapies like heparin and aspirin, intravenous immunoglobulin (IVIg) medication, parental lymphocyte vaccination, and the newly utilised 1alpha, 25-dihydroxyvitamin-D3 (VD3) treatments are all important medications for the isoimmune cause of RSA (Coulam 1991; Lv *et al.* 2018). Their findings are the result of proper humoral factor induction, which results in a transition from Th-1 to Th-2 level, which leads to substantial immune system changes. The function of NK cells is hindered in females with RSA.

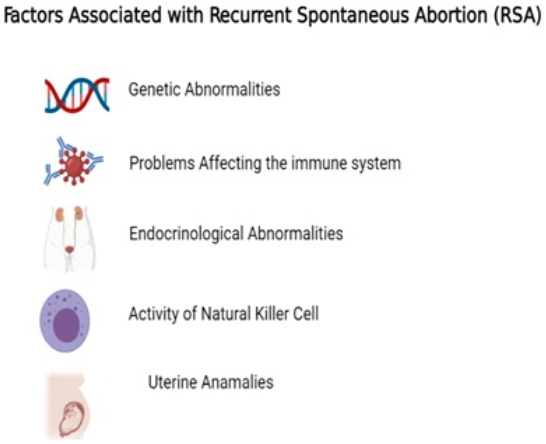

Fig. 2 Factors associated with recurrent spontaneous abortion (RSA)

Anomalies of the chromosomes

According to several studies, de-novo numerical defect such as autosomal trisomy of chromosomes thirteen, fourteen, fifteen , sixteen, twenty-one, and twenty-two, as well as monosomy X, are responsible for the plurality of abortions in women with (RSA). The maternal chromosomal aberration may be caused by a structural mutation such as reciprocal as well as mosaicism of numeric deviations. Several research has discovered that among people with (RSA), skewed X-chromosome blocking (the selective inactivation of one of two X chromosomes in female cells) is extremely high (>90 percent). A chromosomal analysis of the abortion gives an interpretation in at least half of the cases. Parental karyotyping does not predict a potential miscarriage. It is not recommended that couples with recurrent miscarriage undergo routine karyotyping. Since an aneuploidconceptus suggests a slightly higher probability of success with a subsequent pregnancy, cytogenetic analysis may be conducted on products of conception to prevent unnecessary assessment and care (Evidence Level III) (Pandey *et al*., 2005; Wu 2012).

Immunologic Factors

In 30% of women with RSA, autoimmune component represent the mother's activity recognition to conception (self-immune difficulties), which can contribute to foetal rejection. One form of autoantibody that may induce abortion in females with RSA is antiphospholipid antibodies (APA), antinuclear antibodies (ANA), and non thyroid antibodies (ATA) (Lu *et al.,* 2019). Later in pregnancy, antiphospholipid antibodies have been attributed to decidualvasculopathy, thrombosis, and placental infarction. Placentas from females with increased antiphospholipid antibody levels had somewhat more fibrosis, hypovascular villi, thrombosis, and infarction, as well as fewer vasculosyncitial membranes, in a case-control sample of 47 parturiencies that ended in embryo death. Antiphospholipid antibodies have been attributed to a variety of medical and obstetric complications, as well as multiple spontaneous abortions. Anti-phospholipid antibody syndrome (APS) is diagnosed when a patient develops specific quantities of antiphospholipid antibodies thus displaying particular clinical characteristics Antiphospholipid syndrome (APS) and antiphospholipid antibodies have a similar association to other autoimmune diseases and autoantibodies, such as systemic lupus erythematosus (SLE) and antinuclear antibodies (ANA). These antibodies do not cause miscarriage of their own, but they do damage to a blood vessel's inner wall, allowing blood cells to bind to the injury and form a blood clot. In women with RSA, blood clots and confined blood vessels may reduce blood flow to the embryo and placenta, causing foetal death or growth retardation (Pandey *et al*. 2005).

Endocrinological Disorders

Polycystic ovary syndrome (PCOS), luteal phase disorder, thyroid disease, as well as diabetes mellitus are also endocrinological diseases linked to RSA. The most frequent cause of PCOS is ovarian hyperandrogenism, which is thought to be triggered by a primary dysfunction in androgen biosynthetic pathway (Li *et al*., 2017). Obesity and hyperinsulinemia are two of the most common manifestations. PCOS is very common in patients with RSA, with rates ranging from 44 to 82 percent during ultrasound examination (Baptiste *et al*. 2010). This syndrome causes excessive luteinizing hormone (LH) secretion in women. Increased LH secretions can have a detrimental impact on development of oocyte or endometrium, either actively or passively, by increasing testosterone as well as oestrogen levels. PCOS increases the risk of abortion in women by 25–40% (Jeve *et al.,* 2014). The most successful therapy is to increase follicle stimulating hormone (FSH) to induce ovulation. Insufficient endometrial

maturation causes luteal phase deficiency in women with RSA, resulting in a qualitative as well as quantitative malfunction in corpus luteum function and insufficient progesterone output. In one study, women with thyroid autoantibodies had a 17 percent chance of having an abortion compared to 8.4% in the control group. In insulin-dependent diabetics, however, the risk of miscarriage in the first trimester is 15 percent (with successful glucose tolerance) and 45 percent (with bad glucose tolerance) (Zolghadri *et al*. 2008). The embryotoxic effects of hyperglycemia may contribute to a higher rate of pregnancy loss in women with impaired glucose tolerance. On the other hand, women with RSA with pregnancy failure in the second trimester or third trimester as well as clinical symptoms of diabetes mellitus should have their glucose tolerance tested.

Activity of Natural Killer Cell

Natural Killer cells play a critical role in immune response and control by influencing Non-MHC- restricted cytotoxic effects on target tissue.High presumptional peripheral Natural Killer cell activation has been linked to subsequent abortion in women with RSA. NK cell function in the peripheral blood is greatly reduced during early pregnancy in comparison with the non - pregnant condition. Absolute Natural Killer cell action drops in the third month of gestation and then rises during the postpartum cycle in comparison to non-pregnant condition. Females with RSA who share HLA DQ A1 genotypes with their partners have more CD56+ cells in their accessorial blood than women who have regular pregnancies, according to research on peripheral blood Natural Killer cells. When luteal phase endometrial samples from females with RSA and normal cases were evaluated, the endometrium of females with RSA had a higher number of CD56+ cells. In comparison to women who had healthy live births, endometrial diagnostic tests of several women with RSA revealed a significant increase in multiple cell populations (CD4+, CD8+, CD14+, CD16+/CD56+). Women with RSA, on the other hand, had a higher proportion of CD56+/CD16 cells in their deciduas, which contain suppressor factors and cytokines (40%) than women without the disease. Activation of parental NK cells has been related to subsequent abortion in females with normal genes, according to some reports. Another hypothesis for RSA-related foetal loss is that Th-1 cytokines activate NK cells, resulting in foetal loss. As a result, blocking NK cell growth in the foetus is likely to inhibit the maternal alloimmune response in the foetus (Motedayyen *et al*. 2018; Zhu *et al.,* 2021).

Anomalies of the uterus

Uterine anomalies such as septate uterus, bicornuate uterus, and uterus didelphys, which are caused by imperfect integration of Mullerian ducts, are often associated with (RSA). Cervical incompetence was found to be more prevalent in females with a bicornate uterus (38 percent). Late miscarriage, immature delivery, and minor mullerian (hypoplastic and arcuate uterus) anomalies are one of the most prevalent anatomic uterine anomalies, which range from 1.8 to 37.6%. Because cervical cerclage is the most effective treatment for a bicornuate uterus, surgical correction (metroplasty) is only an option for women who have experienced multiple late-trimester losses or prematurity. Of all the big mullerian abnormalities, a didelphic uterus has the greatest prognosis. Patients with (RSA) can benefit from hysteroscopic resection to remove intrauterine adhesions. One of the more common hysterogram anomalies is a narrow T-shaped uterus with cavity constrictions. Hysteroscopic lateral metroplasty can help women with RSA avoid miscarriage. Cervical incompetence, which is caused by a sudden rupture of membranes followed by a painless abortion, is one of the most common reasons of second trimester miscarriage. Cervical cerclage is the standard

treatment in these cases. Its effectiveness, on the other hand, is debatable (Pandey *et al*. 2005).

Methods for treatment of recurrent spontaneous abortion

Therapy dependent on natural killer cells (NK cells)

Natural Killer activation receptors (NKp46, Fc RIIIa) can be blocked to boost pregnancy outcomes. The research shows that dNK cells play an important physiological role in maintaining a healthy placenta. Excessive or insufficient dNK activity may lead to pregnancy complications like RSA, preeclampsia, and Fetal growth restriction. Despite this, women now have access to a number of treatments aimed at reducing Natural Killer cells (Hao *et al*., 2020). This approach is based on the incorrect premise that a massive proportion or behaviour of Natural Killer cells is linked to bad pregnancy outcomes. If blood tests for pNK cells are elevated, women are given a variety of therapies, including prednisolone, intravenous Ig, Intralipid, and TNF-blocking therapeutics. NK cell-based treatment is now extensively used in cancer treatment. To reverse impaired foetal development, they adoptively transfer Natural Killer cells in a noninvasive manner, such as an intravenously or a genital suppository. NK cells are superior to pluripotent stem cells in that they are less likely to grow into tumours (Liu *et al*., 2019).

Aspirin or heparin therapy

Women who become pregnant after receiving preconception treatments and also have APA and increased Natural Killer cells have a live birth rate of around 70%. The first line of defence is normally low-dose heparin and aspirin treatment. Heparin is a large enzyme that does not pass through the placenta, but aspirin does (Lu *et al*., 2019). Normal placentation requires inflammation, and chorionic villi anchoring is aided by activation of coagulation pathways. Antiabortogenic medicines like heparin and aspirin look counterintuitive if inflammation and blood clotting play a role in the foetus' survival. Prednisone, which inhibits the inflammatory reaction and settles down the cell, can also be used to treat antinuclear antibodies.

Intravenous immunoglobulin (IVIg) therapy in RSA

IVIg therapy is a safe care choice for women who have missed a baby due to an immunological cause caused by heparin or aspirin use. On the newborn, IVIg's main function is to counteract the cytotoxic activity of the paternal immune response. According to several reports, antiphospholipid antibodies inactivate idiotype-bearing B cells, reduce cytokine formation, and suppress complement activation. Antiphospholipid antibodies are passively transmitted blocking or antiidiotypic antibodies that prevent antiphospholipid antibodies from binding to related antigens (Muyayalo *et al*., 2018).

Immunotherapy for Increased Circulating CD56+ Cell Concentrations Associated with Recurrent Spontaneous Abortion

T regulatory cells (Tregs) are a type of CD4+ CD25+ T cell that helps to prevent autoimmunity and promote transplantation tolerance (Qian *et al*., 2018). Flow cytometry and triple immunofluorescence marks were used to examine the proportions of lymphocyte subsets in PBL and deciduae, as well as FOXP3 expression in those cells, in URSA patients, typical early pregnant women, and average nonpregnant women (Motedayyen *et al*., 2018; Zhu *et al*., 2021). According to the findings, CD4+ CD25+ high and CD4+ CD25+ low cell concentrations in PBL are statistically significantly higher in URSA patients with early abortion and normal early pregnant women than in nonpregnant women in URSA

patients with early miscarriage (Mei *et al*., 2010). The FOXP3 gene, an essential regulatory gene, is required for CD4+ CD25+ Treg cell development and function. FOXP3 expression was found in the majority of CD4+ CD25 + high cells, with lower levels in CD4+ CD25 + low cells and almost negligible expression in CD4+ CD25+ T cells, according to their findings. Somerset's findings backed up this theory Mei *et al*. 2010) (Somerset *et al*. 2004). This means that CD4+CD25+high T cells are required for a healthy pregnancy and that their loss may contribute to the development of URSA (Motedayyen *et al*. 2018; Zhu *et al*. 2021). In URSA patients, lower FOXP3 expression is linked to a decline in CD4+CD25+ high T cells Mei et al. 2010; Sereshki *et al*., 2014; Quan and Yang 2017; Roomandeh *et al.,* 2018 and Hao *et al*. 2020).

Conclusion

A patient who experiences a recurrent spontaneous abortion should be recognized. Because recurrent spontaneous abortion affects such a wide range of women, particular markers are needed to determine which ones will respond to different treatments. In this review we found that a variety of genetic, anatomic, endocrinologic, microbiologic, immunologic, and metabolic factors have been related to recurrent spontaneous abortion. Should be recognized, as it possess serious healthcare problem. Treatments that are both safe and successful are needed. Testing that have been accepted as standard practise and are used on a regular basis should be reevaluated to see if the test result has an impact on the pregnancy outcome. It is therefore necessary to perform tests that have not yet been accepted as standard. Early chorionic villus tissue sampling and karyotyping showed that a large proportion (on average 60%) of failing pregnancies have chromosomal abnormalities. In those aborting karyotypically normal embryos, peripheral blood NK cell levels (and presumably endometrial NK cell levels) tend to be elevated. Active therapies with maternal mononuclear cells and passive immunotherapy with IVIg are two treatments that may help affected couples have a live birth. The fact that both therapies decrease NK activity in vivo while increasing immunosuppressive activity, which prevents NK cell activity, indicates a possible mechanism. Identifying the immunologically mediated pathways linked to recurring spontaneous abortion and treating each patient appropriately remains a challenge.

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RESEARCH ARTICLE

Nitrogen and Carbon Dynamics in Brassica under Elevated CO_2 and Moisture Stress Condition

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Abstract

A study was carried out to see the effects of elevated levels of CO_2 on partitioning of Nitrogen and Carbon in two cultivars of Brassica species viz. RH-30 and Pusa Gold under moisture stress condition. Results revealed that elevated CO_2 increased the C content of the leaves, stem and root;however, there was significant reduction in N content in various part of the plant at various stages of growth which could be linked to the overall alteration plants C: N ratio. Betweenthe two cultivars higher C content was recorded in RH-30 cultivar. The higher level of C was also recorded in elevated CO_2 of 550 ppm and under moisture stress conditions, indicating that crop growth under 55O ppm of CO_2 has some amelioration effect when compared to ambient. More accumulation of carbon may result in the better root growth, root number (both secondary and tertiary) and volume of root under due to elevated CO_2 which may further help in the absorption of available water and nutrients. The higher level of carbon content was not only recorded in the leaf during flowering, but also recorded in the pre-flowering stage indicating that C could be used as a storage carbon during reproductive development under the elevated CO_2 condition.

Key words: Brassica, Carbon, Dynamics, Moisture, Stress, Carbondioxide, Nitrogen, RH-30, Pusa Gold.

Introduction

Emissions of greenhouse gases have increased rapidly in the last centuries, particularly in the last few decades, due to human activities (IPCC, 2014). As a result of these effects, higher levels of atmospheric CO_2 have altered the carbon sequestration pattern in the ecosystem and influenced plant growth, resulting in a change in the dry matter accumulation partitioning pattern. Various workers reported that Plants are especially responding to climate change in various ways like photosynthetic efficiency (Das 2020b) in Brassica, reproductive development (Das *et al*., 2021) in chili, plant architecture (Gray and Brady, 2016; Sage *et al*., 2008), plant anatomy (Das, 2021a) in Brassica, crop phenology (Das *et al*., 2020 in chili; nutrient uptake (Wang *et al*., 2019 in rice and wheat) since their performance is largely dependent on environmental conditions and ultimately effect on crop productivity. So, Climate change is now considered as global threat to the food and nutritional security of the world (Malhi *et al*., 2021).

The sink strength, which is mediated by N supply, is critical in regulating how plants respond to rising CO_2 levels in the atmosphere (Allen *et al*., 1988). Higher CO_2 resulted in an increase in NUE and a decrease in N, S, and B concentrations per unit tissue (Wong, 1990) and ultimately Changes in nutrient uptake and utilization under simulated climate change conditions (Wang, *et al*., 2018) in rice. Depending on the nitrogen

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availability in the soil, the N content of foliage and roots was consistently lower in plants grown at high CO_2 (Hocking and Meyer, 1990).When N is readily available, any influence of CO_2 on shoot N concentration virtually disappears, indicating the presence of an uptake limitation, probably related to the flux of NO_3- to the root surface (Vassey *et al.*, 1991). Reduced tissue N concentrations in plants grown in high CO_2 conditions could indicate physiological alterations in the efficiency with which plants utilize N to gain biomass (Hilbert *et al.*, 1991). According to Conroy (Chaves and Pereira, 1992) high CO_2 affects nutrient requirements and N concentrations in leaves, roots and grains are consistently lower in plants grown at elevated CO_2, regardless of soil N availability. Ziska *et al.* (1996) found that in rice grown at ambient and elevated CO_2 settings with three levels of N supplementation, photosynthesis became dependent on N in later growth stages. Furthermore, in cotton, the leaf N concentration was to be reduced by 33 percent on a total dry weight basis when grown at a CO_2 concentration of 550 µmol mol-1 compared to those grown at ambient CO_2. As the N supply was increased, the CO_2 effect declined until there was no significant reductions in leaf N concentration at the highest N supply (133 mg N kg-1 soil week-1). The observed reduction in leaf N concentration was partly due to dilution of leaf N by non-structural carbohydrate, but it was not entirely due to it (Rogers *et al.*, 1999)

According to Uprety and Mahalaxmi (2000), higher CO_2 enhanced carbon sequestration in Brassica plants' leaves, stems, and roots by increasing photosynthesis and decreasing respiration. Excess carbohydrates, according to them, make plants more flexible and responsive to extra N application, allowing them to sustain this CO_2 enrichment. Plant and soil-related regulation of C and N interaction in the rhizosphere are still poorly understood and require classification (Griffin *et al.*, 1993). As a result, forecasting plant response to higher levels of atmospheric CO_2 necessitates an understanding of the interaction between CO_2 and limiting environmental factors such nutrient availability, particularly N. The critical aspect from an agricultural standpoint is to understand how simultaneous increases in CO_2 and temperature would influence various plant groups (Kaciene *et al.*, 2017). In this study, we want to elucidate whether the increasedlevel of CO_2 will alter the partition pattern of C and N in various stages of growth in Brassica under moisture stress conditions. To explore this idea, we conducted an experiment in a simulated environment with elevated CO_2 and moisture stress, to study how these two variables interactively affect the partitioning and accumulation of N and C.

Material and methods

Plant Material

Brassica cultivars viz. *Brassica juncea* cv. RH-30 and Brassica campestris cv. Pusa Gold were collected and grown for the present investigation.

Experimental site and growth conditions

The response of both thespecies to elevated CO_2 was studied using Free Air CO_2 Enrichment Technology (FACE) to simulate the doubling CO_2 concentration at, IARI, New Delhi-12. The crops were grown in the field and inside the Mid Free Air CO_2 enrichment (FACE) facility in 8 m diameter circles. An elevated CO_2 concentration of 550 µmolmol-1 was maintained throughout the crop growth period with the help of computer-based PID valves. There was no exogenous supply of CO_2 to the normal air under ambient field condition. Field was prepared by recommended agronomic practices.

Cultural practice

Farmyard manure was applied at the rate of 5 tons per hectare at the time of field preparation. The plant spacing, fertilizer application at the rate of 30+30:60:40 kg per hectare of nitrogen, phosphorus and potassium and other cultural practices were followed as reported by Uprety *et al.*, (2001).

Moisture stress treatment

Moisture stress treatment was given by restricting irrigation and bringing the soil moisture level between 7 and 10% compared to 22-25% under irrigated condition. All the observations were taken in triplicate for each treatment at Stage-1: vegetative (25 days after sowing), Stage-2: flower bud initiation (45 DAS), Stage- 3: 50% flowering (60 DAS) and Stage-4: post flowering (75DAS).

Estimation of carbon

Carbon content was estimated by wet digestion following the modified Walkley-Black method (Walkley and Black, 1934). The dried plant samples were oxidized with a mixture of potassium dichromate and concentrated sulphuric acid using heat of dilution of the acid. The unused potassium dichromate was estimated by back titration with ferrous ammonium sulphate. The carbon content was calculated using following formula and expressed in percentage.

Where, A=Weight of the sample X=Volume (ml) of ferrous ammonium sulphate solution required for blank titration, Y=Volume (ml) of ferrous ammonium sulphate needed for the sample. The carbon percentage was obtained by multiplying this C per cent with a constant, 1.3, as only 77 per cent recovery was presumed from oxidation of sample in this procedure.

Determination of nitrogen

Nitrogen content in plant parts was measured by Kjeltec Auto 1028 Analyzer. The dried plant material was digested with concentrated H_2SO_4 in the presence of catalyst to convert the nitrogen to ammonium sulphate. Ammonia was liberated and collected in boric acid solution as ammonium borate by steam distillation of the salt in the presence of a strong alkali which is estimated against a standard acid titration. The dried samples were ground to powdery form. Hundred milligram of sample was transferred to different digestion tubes. To this tube, 10 ml of concentrated sulfuric acid was added. One Kjelatab (containing 3.5 g K_2SO_4 + 12.48 mg CuSO4. $5H_2O$ was added to the digestion tube. An assembly of 20 digestion tubes were put in digestion block and covered with fume hood. The digestion temperature was raised to 400oC till the samples turned turquoise (about 1 hr). The digestion tubes were removed and allowed to cool for 5-10 minutes. About 50 ml of distilled water was added and mixed well.

The digested extract was made up 100 ml with distilled water. The tube was then fitted to the splash heat for distillation. The instrument was operated as per Tecator (1985) manual. When the distillation and titration cycle is over, the result is displayed on the front panel.

Prcent N is calculation

, Vsample = the volume (ml) standard HCl for titration of the sample, VBlank = the volume (ml) standard HCl for titration of the sample, M= molarity of solution, g sample= dry weight of sample

Results and Discussion

Carbon content in different parts

Leaf

The carbon content in the leaves of Brassica cultivars was significantly more in plants grown under high CO_2 **(Fig. 1 a-f)**. This increase ranged from 35% (vegetative) to 38% (flowering). Carbon content was more in the leaf of 'RH-30'. Moisture stress treatment significantly decreased the carbon content ranging from 47% (vegetative) to 60% (flowering and post flowering). The reduction under ambient and elevated CO_2 conditions varied between 37% (flowering) to 43% (post flowering) and 23% (vegetative) to 34% (flower bud initiation) respectively in 'Pusa Gold', whereas, in the case of 'RH-30' the reduction ranged between 31% (flowering) to 38% (vegetative) under ambient condition and between 21% (vegetative) to 28% (post flowering) under elevated CO_2 condition.

Stem

CO_2 enrichment significantly increased the carbon content between 28% (vegetative) to 38% (flower bud initiation) in the stem of Brassica cultivars **(Fig. 1 a-f)**. Carbon content was more in the stem of 'RH-30'. Moisture stress significantly decreased it ranging between 35% (vegetative) to 45% (flower bud initiation). This reduction under ambient and elevated CO_2 condition varied between 30% (post flowering) to 36% (vegetative) and 15% (post flowering) to 26% (flower bud initiation) respectively in 'Pusa Gold', whereas, it ranged between 24% (post flowering) to 33% (vegetative) and 10% (vegetative) to 23% (flowering) respectively in 'RH-30'.

Root

Elevated CO_2 markedly increased the carbon content of roots varying from 22% (flower bud initiation) to 32% (vegetative) **(Fig. 1 a-f)**. Carbon content was more in roots of 'RH-30' cultivar. Moisture stress treatment significantly decreased the carbon in the roots between 33% (post flowering) to 41% (flower bud initiation). This reduction under ambient and elevated CO_2 condition varied between 27% (flowering) to 31% (flower bud initiation) and 17% (flowering) to 23% (vegetative) respectively in 'Pusa Gold' cultivar, whereas, between 21% (post flowering) to 26% (flower bud initiation) and 14% (flowering) to 19% (vegetative) respectively in 'RH-30'.

Nitrogen content of the different Parts

Leaf

CO_2 enrichment significantly decreased the nitrogen content ranging from 12% (vegetative) to 29% (flowering) **(Figure 2 a-f)**. It was higher in 'RH-30' compared to 'Pusa Gold'. Moisture stress significantly reduced the nitrogen content ranging between 19% (vegetative) to 24% post flowering. This reduction under ambient condition varied between 21% (flowering) to 28% (post flowering) in 'Pusa Gold', whereas, in 'RH-30', it ranged from 19% (flowering) to 27% (post flowering) respectively. The interactive effect of CO_2 and moisture stress on nitrogen was not significant.

Stem

CO_2 enrichment markedly decreased the nitrogen content ranging from 17% (vegetative) to 26% (flowering) **(Fig. 2 a-f)**. Nitrogen content of stem was more in stem of 'RH-30'. Moisture stress decreased it significantly between 22% (post flowering) to 27% (flowering). The interactive effect of moisture stress and CO_2 on nitrogen content was not significant.

Root

Nitrogen content in roots of Brassica cultivars was significantly decreased due to CO_2 enrichment varying from 12% (flower bud initiation) 16% (flowering) **(Fig. 2 a-f)**. Nitrogen content was more in roots of 'RH-30'. Moisture stress significantly reduced it ranging from 18% (flower bud initiation) to 22% (flowering). The interactive effect of moisture stress and CO_2 on nitrogen content was not significant.

C/N ratio of different parts of plant:

Leaves

CO_2 enrichment brought about significant increase in the C/N ratio in leaves ranging between 51% (Post flowering) to 72% (flower bud initiation) **(Table 1)**. Varietal difference for this character was not significant. Moisture stress reduced the C/N ratio ranging between 11% (post flowering) to 34% (flowering). The reduction in C/N ratio under ambient and elevated CO_2 conditions varied between 20% (flowering and flower bud initiation) to 34% (vegetative) and 17% (post flowering) to 31% (vegetative) respectively in 'Pusa Gold', whereas, it ranged between 19% (flowering) to 20% (vegetative) and 15% (post flowering) to 21% (vegetative) respectively in 'RH-30'.

Stem

Elevated CO_2 brought about significant increase in the C/N ratio in stem of Brassica ranging from 48% (post flowering) to 67% (flower bud initiation) **(Table 2)**. There was no significant difference in C/N ratio between the cultivars. Moisture stress decreased the C/N

ratio ranging from 10% (post flowering) to 21% (flower bud initiation). The interactive effect of CO_2 and moisture stress was not significant for this character.

Root

Elevated CO_2 brought about significant increase the C/N ratio ranging between 20% (flower bud initiation) to 52% vegetative) **(Table 3)**. There was no significant vertical difference in C/N ratio. Moisture stress significantly decreased it from 11% (vegetative stage) to 46% (flower bud initiation). The interactive effect of CO_2 and moisture stress was not significant for these characters. Senthil-Nathan2021 reported that plant carbon accumulation increased due to elevated CO_2, causing physiological changes that decreased nitrogen content. Similarly, elevated CO_2 increased insect feeding, and did alter other variables such as their biology or reproduction.

Elevated CO_2 changed balance of nutrient by enhancing carbohydrate accumulation in different parts of the plant which may lead to imbalance of other nutrients (Gifford *et. al* 2000 and Yuan *et al.*, 2015). For example lower N concentration (Cotrufo *et al,*. 1998) or enhancement of carbon nitrogen ratio and carbon phosphorus ratios in functional parts of the plant (Sardans *et al.*, 2012). The response of the C:N or C:P ratios to increased CO2 levels, however varied widely between ecosystems (Yuan *et al.*, 2015,).

The plant's C: N ratio is determined by the rate of C and N assimilation and turnover. The C/N ratio suggests that the distribution of resources to secondary compounds is controlled by the carbon-nutrient status of a plant (Wang *et al.*,2019). Under elevated CO_2, the C/N ratio of several plant parts increased significantly. As a result of CO_2 enrichment, this ratio normally tends to rise. The rise in the C/N ratio could be attributed to nitrogen dilution as a result of greater carbohydrate concentrations; a reduction in the requirement for nitrogen in green tissue due to a reduction in Rubisco concentration; the photo respiratory pathway depression due to a lack of enzymes in the glycolate pathway, resulting in lower N demand and; proportionate rise in sink size in the presence of sufficient nutrients.

Present study also indicated that C:N ratio was considerably altered in combination with elevated CO_2 and moisture stress, which significantly differed from ambient level of CO_2 and moisture stress. Fernandoet al.,2014 and Gunderson *et al.*, 2000 also reported the similar effect according to them elevated CO_2 offsetor ameliorated the effects of moisture stress. Increase in C:N ratio under elevated CO_2 with irrigation as well as under moisture in combination with elevated CO_2 indicates that the positive efect of elevated CO_2 on C:N ratio might have compensated for the negative impact of moisture stress. These changes were recorded in the present study and conformity with results of Upretyet al., 2002 in rice. Conroy and Hocking (1993) opined that an inadequacy of N assimilates partitioning was responsible due to the deficit of N. The propensity for CO_2 enrichment to raise the C/N ratio resulted in limitation of nitrogen in plantbut that can be overcome by (Riviere-Rolland *et al.,* 1996 and Uprety and Mahalakshmi, 2000).

Similarly, Wang et al., 2019 reported that elevated CO_2 enhanced the C : N ratio by 8–14% in rice-winter wheat rotation system. More accumulation of carbon may result in the better root growth, root number and volume of root under due to elevated CO_2 which may help in the absorption of available water and nutrients. The increase in these parameters helped in the absorption of available water variation in responses C partitioning into different organs was also recorded in grapevine Tempranillo clones under simulated climate change scenarios like elevated CO_2 and temperature (Arrizabalaga-Arriazua *et al.*, 2020). Leaf carbohydrate concentration was related to increase structural C to N ratio and thus influence an increase of C simultaneously with N reliant sink limitation under elevated CO_2 (Fischer *et al.*, 1997).

Habeisen *et al.* (1997) in his studies observed that the response of yield in L. perenne was rely on nitrogen fertilizer, when crop grown under Free air enrichment technology. Similarly elevated CO_2 improved the partitioning of biomass to the roots, which provide as powerful sink for assimilated carbon (Rabha and Uprety, 1998).

According to Ainsworth and Long (2005) the chemical composition of plants showed variation when the C/N ratio of the plants changed under elevated temperatures. This changecould be due an increase in photosynthetic activity thereby increasing the leaf carbohydrate (sugars and starches) per unit leaf area under elevated CO_2.According to them, increased CO_2 causes a decrease in leaf N concentrations in plants, which is often associated with a decrease in N per unit leaf mass.They also opined that the decrease in N in tissue may be due to dilution of N because of greater accumulation of non structural carbohydrate; due to decreased stomatal conductance, mineral uptake from the soil may be reduced, and plants may take up less water (Wang *et al.*, 2018) and consequently, with time, decline in nitrate absorption into organic molecules (Bloom *et al.*, 2010).

Concentrations of protein in the plant tissues are related to the N status of the plant tissue so, alteration in N in plant tissue have significant effects on plants at elevated levels of CO_2. Interestingly a decrease in N content in tissue under elevated CO_2 is very less as legumes fix N from atmosphere in comparison to other C_3 species (Cotrufo *et al.*, 1998; Jablonski *et al.*, 2002; Taub *et al.*, 2008, Aziz *et al.*, 2020). Increased CO_2 altered root morphology, resulting in an increase in fine root proliferation, which is expected to improve plant nutrient absorption (Bentley et al., 2013; Beidler *et al.*, 2015).

From the present investigation it was clear that the accumulation of C in various plant parts viz. roots , stem and leaf was considerably more in higher level of CO_2. The exceptionally higher C content was observed in the leaf during flowering as compared to other stages of growth in elevated level of CO_2 condition that suggesting that it may serve as a storage site for carbon and being transferred to the sink zone during reproductive stage ultimately helps in siliqua formation

Table 1. Interactive effect of elevated CO_2 and moisture stress on C:N ration in leaves of *Brassica species*

Treatments	Vegetative		Pre flowering		Flowering		Post flowering	
	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30
FACE IRR	8.77	10.00	9.90	8.45	13.26	15.89	9.08	8.41
FACE MS	7.84	8.99	6.820	6.64	10.69	13.01	7.440	6.94
AMB IRR	6.39	6.38	6.85	6.04	7.53	10.58	4.630	6.140
AMB MS	5.19	5.25	4.53	4.52	6.00	8.48	3.70	4.97
Var.	NS		NS		NS		NS	
CO_2	0.371		0.351		0.627		0.678	
Var. x CO_2	0.525		0.497		0.805		0.978	
MS	0.454		0.592		0.539		0.336	
Var. x MS	0.642		0.722		0.624		0.475	
CO_2 x MS	0.743		0.891		0.762		0.524	
Var. x CO_2 x MS	0.908		1.184		1.078		0.673	

Table 2. : Interactive effect of elevated $CO_2$and moisture stress on C:N ration in stem of *Brassica Species*

Treatments	Vegetative		Pre flowering		Flowering		Post Flowering	
	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30
FACE IRR	10.05	10.96	10.00	10.48	13.09	11.28	10.72	10.39
FACE MS	8.26	9.23	7.79	8.32	10.74	9.55	9.56	9.30
AMB IRR	6.98	7.91	5.58	5.50	6.71	6.64	7.22	6.35
AMB MS	6.46	7.40	5.28	5.21	6.21	6.02	6.77	6.35
Var.	NS		NS		NS		NS	
CO_2	0.609		1.12		1.78		1.01	
Var. x CO_2	0.862		1.89		2.14.		1.89	
MS	0.544		0.98		0.23		0.330	
Var.×MS	0.612		1.14		0.54		0.476	
CO_2 x MS	0.770		1.77		0.79		0.512	
Var. x CO_2 x MS	1.089		2.01		1.24		0.732	

Table3.: Interactive effect of elevated $CO_2$and moisture stress on C:N ration in roots of *Brassica species*

Treatments	Vegetative		Pre flowering		Flowering		Post flowering	
	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30	Pusa gold	RH-30
FACE IRR	13.78	13.88	20.67	17.57	18.74	18.19	19.52	16.76
FACE MS	11.09	11.40	16.37	14.84	16.36	16.04	16.05	13.85
AMB IRR	8.53	8.20	13.14	11.00	12.23	10.93	12.41	11.10
AMB MS	8.11	7.77	12.33	10.05	12.01	11.02	11.95	10.76
Var.	NS		NS		NS		NS	
CO_2	0.475		2.33		1.12		0.702	
Var. x CO_2	0.672		4.01		2.33		1.13	
MS	0.409		0.88		0.63		0.544	
Var. x MS	0.578		1.01		0.91		0.067	
CO_2 x MS	0.919		1.67		1.13		0.770	
Var. x CO_2 x MS	1.34		2.04		1.89		1.08	

FACE = Free air CO_2 enrichment, IRR= irrigated, MS = Moisture

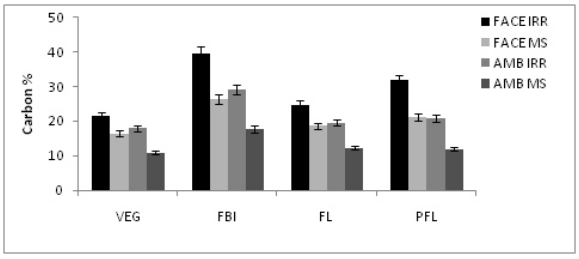

Fig. 1 (a) Interactive effect of elevated CO_2 on carbon content of leaf at various stages of growth in Pusa Gold

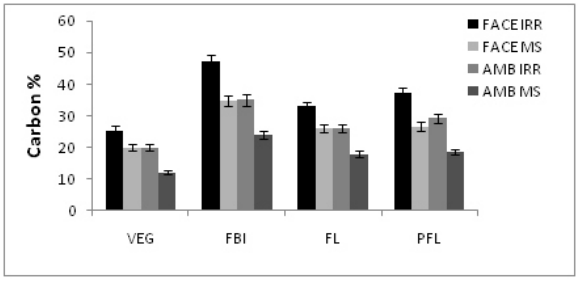

Fig. 1 (b) Interactive effect of elevated CO_2 on carbon content of leaf at various stages of growth in Pusa Gold

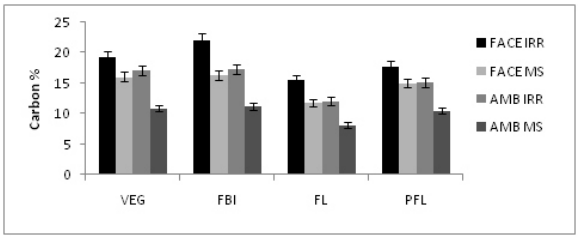

Fig. 1 (c) Interactive effect of elevated CO_2 on carbon content of stem at various stages of growth in Pusa Gold

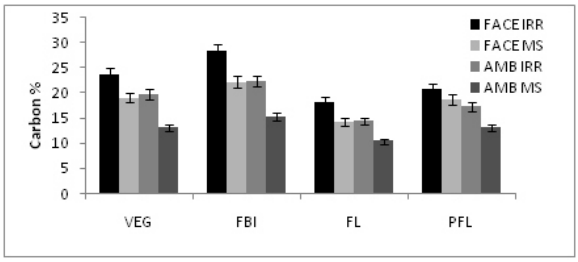

Fig. 1 (d) Interactive effect of elevated CO_2 on carbon content of stem at various stages of growth in Pusa Gold

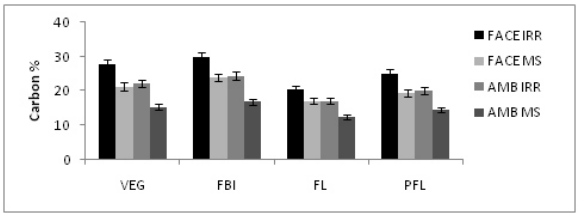

Fig. 1 (e) Interactive effect of elevated CO_2 on carbon content of root at various stages of growth in Pusa Gold

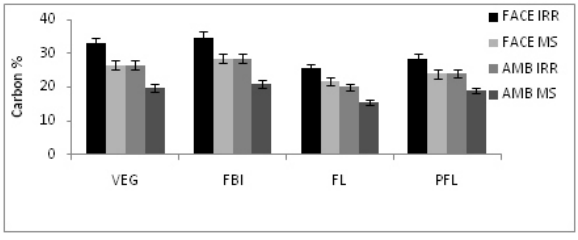

Fig. 1 (f) Interactive effect of elevated CO_2 on carbon content of root at various stages of growth in Pusa Gold

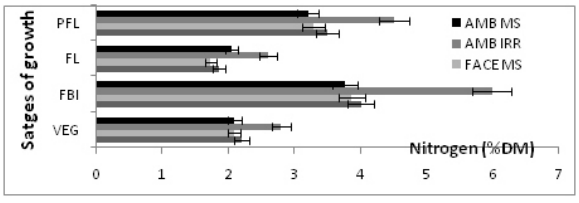

Fig. 2 (a) Interactive effect of elevated CO_2 on N content of stem at various stages of growth in Pusa Gold

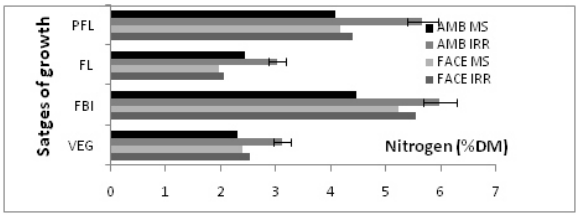

Fig. 1 (b) Interactive effect of elevated CO_2 on N content of stem at various stages of growth in Pusa Gold

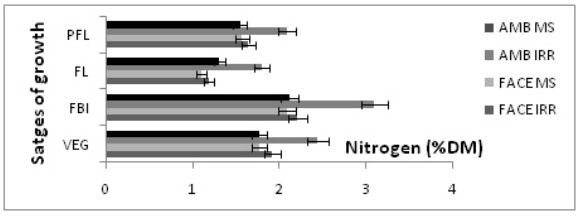

Fig. 2 (c) Interactive effect of elevated CO_2 on N content of stem at various stages of growth in Pusa Gold

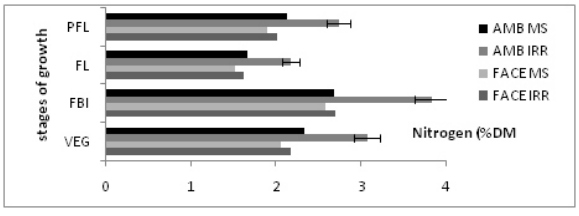

Fig. 2 (d) Interactive effect of elevated CO_2 on N content of stem at various stages of growth in Pusa Gold

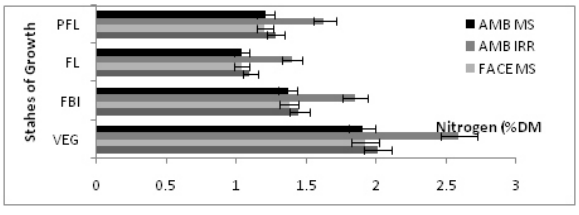

Fig. 2 (e) Interactive effect of elevated CO_2 on N content of root at various stages of growth in Pusa Gold

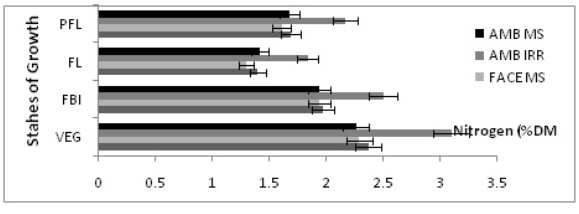

Fig. 1 (f) Interactive effect of elevated CO_2 on N content of root at various stages of growth in Pusa Gold

Conclusion

A clear change was observed in the chemical composition of the leaves, with more carbohydrate accumulation and a decrease in nitrogen concentration in plant as whole. Low concentration of Nitrogen in various part plant in comparison of carbon may be resulted from reallocation of Nitrogen for maintenance of Rubisco enzyme, dilution effect N because greater acumination carbon.

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REVIEW ARTICLE

A Review on Microbial Intervention for Improving Phosphorus Use Efficiency in Pigeon pea-Wheat Cropping System

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Abstract

Efficiency of applied Phosphorus (P) used by the crops varies from 10-30% in a year and the remaining 70-90% turns out to be a part of the soil P pool which is discharged to the crop year by year. The pool supplies to the future crop production enhance the fertilizer efficiency by improving crop recovery in the year in which it was applied and could potentially improve yield of the crop and returns. P is an essential element of all life and is also necessary for worldwide food security. Being it a limited resource, making it efficient use is vitally important. It is normally believed that Phosphorus fertilizer is not efficient because the annual recovery of phosphorus by crops in which it is sprayed is around 10-15%. The unrecovered residual P fertilizer by the crop is supposed to be permanently fixed in the soil in unavailable forms to the plants. However, field experiments don't agree that view. Phosphorus use efficiency(PUE) can be evaluated in many ways, but "balance method" (i.e. fractional nutrient balance) is found to be improved as it is calculated as P removal-to-input ratio. When P is determined by the balance method, P recovery is generally in the series of 50-70% or even higher. Improving fertilizer P use and efficiency is achieved through the implementation of the best fertilizer management practices within circumstance of 4 R- application of the "Right nutrient resource, applied at Right time, Right rate and at Right place." Agricultural production is frequently limited with low phosphorus availability. There are three strategies outlined by which microorganism and plants can improve PUE- root foraging strategies, P- mining strategies and improving the internal P utilization efficiency.

Keywords: Phosphorus management, Dry Matter (DM), Phosphorus fertilizer efficiency, Phosphorus removal-to-use ratio, Partial nutrient balance (PNB)

Introduction

Wheat (*Triticum aestivum*) is the main cereal crop in India. India ranks second largest wheat-producing country. Scientifically called Cajanus Cajan, Pigeon Pea belongs to the familyofpulses. In India, Pigeon Pea is also known as "Arhar",Tur dal, or split pigeon pea, is one of India's most popular pulses and an essential source of protein in a predominantly vegetarian diet. Phosphorus is the very important essential nutrient for cereals and pulseproduction. Phosphorus (P) fertilizer usage for crop output has helped to satisfy food security needs as the world's population has grown.In order to obtain large grain yields, adequate soil P content in the root zone is necessary. Phosphorus (P) shortage, on the other hand, decreases agricultural productivity by more than two billion hectares worldwide.

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Because global Phosphorus resources are insufficient and P fertilizer prices are rising, this shortfall will worsen. As a result, large volumes of Phosphorus fertilizer were applied in the last century to assure adequate P nutrition delivery in soils with low Phosphorus fertility. Low phosphorus availability restricts plant development on a variety of soils from across the world, and it is a major barrier to agricultural output. Phosphorus fertilizer availability is restricted in underdeveloped nations (Lynch, 2007). Phosphorus fertilizers, which are made from phosphate rock, are employed in demanding agricultural organizations to compensate for Phosphorus deficit, and so contribute significantly to world food security and production. Phosphorus reserves in rock are finite and non-renewable; therefore there is worry about the more sustainable use of phosphorus sources in agriculture, as well as the need to enhance phosphorus efficiency (Bouwman *et al*. 2009; Cordell *et al*. 2009; Van Kauwenbergh 2010). Phosphorus (P) is a essential nutrient for plant development and growth, accounting for around 0.2 % of the dry weight of a plant. It is only second to nitrogen as the mineral nutrient that most usually limits crop development (Guignard *et al*., 2017). The phosphorus substance of soil is typically around 0.05 percent (w/w); however, only 0.1 percent of this phosphorus is accessible for plant usage (Zhu *et al*., 2011). Phosphorus fertilizers have usually been used to address the problem of soil phosphorus insufficiency. Plant nutrient uptake is aided by soil microbes. They play an important role in a variety of biological activities, including the transformation of insoluble soil nutrients. (Babalola and Glick, 2012a). For plant growth, some have the capacity to solubilize and mineralize insoluble soil phosphorus. Apart from chemical fertilisation, the only technique to enhance plant-available phosphorus is by microbial P-solubilization and mineralization. In the natural ecosystem, several microorganisms in the soil and rhizosphere are capable of solubilizing and mineralizing phosphorus from total soil phosphorus. (Bhattacharyya and Jha, 2012). Several soil fungi and bacteria can solubilize phosphorus in vitro, and some of them may be able to mobilize it in plants. (Zhu *et al*.).

Phosphorus Use Efficiency

The amount of total biomass or yield created per unit of P taken in, as denoted by the subscripts PUEt and PUEy, according to Hammond *et al*., 2009 Use of Phosphorus Efficiency is defined as the amount of total biomass or yield generated per unit of P taken in. When it comes to biomass, it's common to limit measurements to plant parts that are above ground. In grain crops, PUE is the grain yield per unit of maximum aboveground plant Phosphorus.The effectiveness with which it is employed in growth and metabolism, as well as the duration of its presence in live plant portions where it contributes to these activities, define the productive use of a unit of P taken up in vegetative plants (Verma *et al*). When the seed P stores are spent, the plant's ability to grow depends on root uptake of Phosphorus from the soil. Because fertilizers are freshly applied and there is no resource rivalry among roots, this stage of development usually correlates with exponential growth in agricultural plants, when soil P availability is thought to be at its peak. Even if soil water and nutrients are not depleted, crop relative growth rate will decrease due to self-shading, increased respiratory expenditures, and senescence of older plant tissues and organs. Root P absorption continues at this stage, although remobilization of P from senescing tissues can become a significant inside source of P. Because root P absorption decreases as root development of roots slows and available soil Phosphorus decreases remobilization of P from senescing vegetative tissues is typically the major supply of P for sink tissues during reproductive development. In crops, a main part of the P in the vegetative sections is re-mobilized from the grain, therefore, the soil

Phosphorus accessibility at this time has a significant impact on the grain yield (Sao *et al* 2021).

Microbial opportunities

Pathogens cooperate and significance role in mediating the accessibility of Phosphorus to plants through a variety of ways, including direct root extension to increase uptake. Microbes can be employed in three ways to boost phosphorus usage efficiency in low-phosphorus soils or to lower the quantity of Phosphorus fertilizer required to optimize production.

i. "Root foraging tactics" that increase soil P availability and enable better yields in low-phosphorus soils, lowers the crucial Phosphorus constraint for plant growth and development. This allows fertilized agriculture to be run at lower Phosphorus concentrations in the plants and this can lowers down the amount at which P collects in moderate to high phosphorus-absorbing soil (Simpson *et. al.,* 2011, Mano Bala *et al*., 2011). The accessibility of soil Phosphorus to plants is influenced by the discharge of exudates from root zone into the rhizosphere, either directly or indirectly (Randall *et al*., 2001).

ii. Acidification of the rhizosphereisal so a problem. Root branching, root hair structures, and root development are all important for effective soil P capture, and has a significant genetic variant exists both on and indifferent plant sp. For Example: Lynch and van Beem (1993) revealed that the root architecture of the bean (*Phaseolus vulgaris*) was sensitive to low P availability, and that genotype-to-genotype variation in this feature has contributed to varying P absorption capability. In maize, (*Zea mays*) the same, identical reactions to root development and lateral root growth, as well as their contribution to Puptake, have been described (Zhu and Lynch 2004) and wheat (Manske *et al*. 2000; Liao *et al*. 2008). Significant change in the length of the original hair and morphology have also found that for some species including barley *Hordeum vulgare*, (beans), soybeans, wheat and white *Trifolium repens* (clover) (Caradus 1979; Yan *et al*. 1995; Gahoonia and Nielsen 1997; Gahoonia et al. 1999; Wang *et al*.2004). In the case of the white clover, the strong heritability of the root hairs length allows selection of this trait in a breeding program (Caradus 1995). Genotypes adapted to low P generates more free radicals in response to stress P and have a greater ability to seek out roots in nutrient-rich surfaces soil layers (Lynch and Brown 2001; Lynch 2005; Verma, N. 2021).

Table:1 Variation in shoot and root PUE (expressed in tissue P concentrations) during vegetative growth ofwheat

P concentration (mg P/gm DM)				
CROP	**PLANT AGE**	**SHOOTS**	**ROOTS**	**REFERENCE**
WHEAT	29	1.04 (Low P)	0.74 (Low P)	
		4.27 (Medium P)	1.79 (Medium P)	Fageria and Baligar (1999)
		4.29 (High P)	2.19 (High P)	
	39	1.57-2.29 (Low P)		Ozturk *et.al* (2005)
		2.80-4.49 (High P)		
	42	2.0-3.1 (Low P)	3.0-6.5 (High P)	Yaseen and Malhi (2009)
		3.1-6.2 (High P)		

iii. **"Soil Phosphorus-mining strategies"** that enhances or improves the desorption, mineralization and solubilization of Phosphorus from cautiously-available pools (i.e Lambers *et. al.* 2010) and slowly mineralizing and resist organic Phosphorus pools in the soils (Richardson *et.al.* 2005, Tripathi *et. al.*, 2014). Mining of P (phosphorus) from agriculture soils is not, sustainable in itself. he fundamental goal of this method, however, is to raise and improve Phosphorus turnover in hardly accessible P pools, as well as to reduce the net buildup of Phosphorus that happens when medium to high P-absorbing soils are fertilized.

Mechanisms of phosphate solubilization:

a) **Organic acids:** PSM is commonly accompanied by a reduction in pH of a medium. According to Vassilev *et al.* 1996 analysis approve the presence of organic acids like lactic, fumaric, glycolic and succinicacid.

b) **Chelators**: Chelation of Cabyoxalic acid facilitates the so-lubilization of insoluble phosphates (Illmer and Sacchinner,1992).

c) **Humic substances**: Humic and fulvic acids are strong chelating agents that efficiently chelate calcium and release hydrogen ions (Singh and Amberger, 1990).

iv. Plants having **"improved internal P-utilization efficiency"** (*i.e.* higher plant yield per unit of Phosphorus absorption) may result in a reduction in the quantity of P (Phosphorus) fertiliser needed for agricultural output.Slow-growing species acclimated to low Phosphorus environments use internal Phosphorus efficiency to an excessive degree (Lambers *et al.,* 2010, Matchado *et al.,* 2018), but is also founded in few plant species used in agriculture (Hill *et al.* 2005). Similarly, in case of intercropped moongbean the phosphorus fertilization influenced the pigeon pea equivalent yield over no phosphorus application (Singh 2013). This may be attributed to enhance in yield of the seed of both component crops (Pigeonpea and moongbean) with phosphorus (P) application. Seed inoculations with Rhizobium, PSB + Rhizobium had improved in the yield of pigeonpea in comparison to inoculation. This could be attributed to the fact that Rhizobium, PSB + Rhizobium helps in promoting of the growth of plant and development, which results in elevated essential nutrient uptake and the higher yields.

In the rhizosphere, microorganisms play an important role in the bio-geochemical cycle of organic and inorganic phosphorus (Whitelaw 2000; Harvey *et al.,* 2009; Khan *et al.,* 2010; Richardson and Simpson 2011; Daniel *et al.* 2017), with a considerable percentage of entire culturing bacteria and fungus population being reported to have inorganic Phosphate solubilizing activity (Kucey *et al.,* 1989; Bowen and Rovira 1999). The rhizosphere microbial inoculant have been suggested as implements of the integrated nutrient management systems (Harvey *et al.,* 2009; Khan *et al.,* 2010) with a particular focus on their capacity to augment and improve Phosphorus (P) availability for crops (Kucey *et al.,* 1989; Bowen and Rovira 1999, Jakobsen *et al.,* 2005). The introduction of free-living microorganisms that generate non-specific, beneficial Microbial PGPR Inoculants has been a major focus of crop inoculant research. *i.e.* Rhizosphere Phytobacteria and mycorrhizae Fertilizer application strategies Improvements that inhance P solubility i.e. manures and compost Bio stimulation of native microbes for increased P solubilization and mineralization. Technology that can be mass manufactured and has the ability to stay in the rhizosphere to increase soil phosphorus consumption by crop associations with a wide range of plant hosts. (Bowen and Rovira 1999; Harvey *et al*., 2009; Khan *et al*., 2010).

Accessing PUE (P-Use Efficiency)

"Depending on the criterion employed, "nutrient utilisation efficiency" can be measured in a variety of ways (Casman *et al*., 1998). The only direct means of measuring the efficacy of a single application of Phosphorus for phosphorus crops is to identify the Phosphoric fertilizer with the radio-isotope 32P. As a result of the high cost of this technology and the fact that 32P has a half-life of just 14.3 days, most research are short-term. Crop improvement efficiency (RE) of added phosphorus is called as "the difference method", which is a very frequent and broadly used method to estimate its efficiency. It is calculated as-

$$RE = \frac{UP-U0}{Fp}$$

Whereas, Up-U0 = A crop absorbs P from soils with (Up) and without (U0) additional P.

Fp = amount of P applied

The amount of Phosphorus taken up by a crop grown on soil with no additional P (i.e. the soil's plant-available P) determines the recovery (Snijesh *et al*., 2014). P recovery values using the "difference method" are about 10-15%. These low results create the appearance that the phosphorus supplied is inefficiently used. Many variables impact P-uptake, including nitrogen (N) provided, soil moisture, soil structureand rooting patterns and they should be taken into account when assessing phosphorus efficiency.

If just a limited quantity of Phosphorus(P) is taken up by the crop directly from the fertiliser supplied, the rest must come from soil Preserves, such as P naturally contained in the soil, soil (OM), or manure, or P

accumulated from prior fertilization treatments, such as residual Phosphorus (Syers *et al*. 2008). Reported that replacing the Phosphorus taken up from the soil Preserve was equally as efficient as freshly applied P as that taken directly from fertilizer by the crop. It is expressed as a percent, P revival by this technique is also known as "partial nutrient balance" (PNB) explained by Syers *et al*. (2008) as the **"balance method".**

$$PNB \quad \frac{UP}{FD}$$

"Phosphorus (P) in the crop (Up, part directly from the applied Phosphorus and part from the soil reserve) and Phosphorus applied as fertiliser (Fp) is considered in the term balancing. This procedure is known to as the "P removal-to-input ratio" when presented in a ratio".

Another way of measuring efficiency that incorporates production is partial factor productivity (PFP). PFP measures how productive a crop is in relation to its nutrient intake and is measured in units of crop yield per nutrient applied. It is expressed below as-

Whereas, Y = yield of harvested crop

Fp = P fertilizer applied

Conclusion

Phosphorus (P) is the second most essential macronutrient for plant development, and a scarcity of plant-available P prevents plant growth on about 5.7 billion hectares of land throughout the world. As acid soils, large quantities of P are "locked up" in recalcitrant organic P fractions or non-labile inorganic P pools in complexes with iron and aluminium, as alkaline soils, considerable quantities are "locked up" in resistant organic P fractions or non labile inorganic P pools in calcium complexes. While phosphorus fertilizer can assist farmers overcome these constraints, resource-constrained farmers in under developed countries are frequently unable to do so due to a the high expense of importing and shipping P fertilizers, as well as a lack of locally available P fertilizer sources.(Wissuwa, 2001). Phosphorus fertilizer is most commonly unbalanced because its recovery by the crops is very limited and is about 10-15%. Recovery of phosphorus by the crops is influenced by how the Phosphorus is managed and by suitable application of the 4R – right time, right place, right rate, and right source. Though, microbial intervention may increase the phosphorus use efficiency (PUE) to some extent.

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RESEARCH ARTICLE

Study of Air Analysis to Access Impact of Pollution on Ambient

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Abstract

The present work pertains to analyzing ambient air quality of the study area amidst latitude 29^0 45' 30" N and 29^0 47' 30" N and longitude 79^0 44´E and 79^0 46´E. Depending upon the prevailing wind direction, atmospheric pollution in the area can be attributed to the emissions from the Dead Burnt Magnesite Plant. Besides, dust from mining areas also results in air pollution. The burning of raw magnesite in the shaft kilns has generated emissions which mainly consists of oxides of SO_2 and NOx with heavy load of Suspended particulate matter (SPM) of oxides of Mg, Si, Fe, Al, Ca. These emissions along with dust generated from mine area have affected vegetation around the plant area. While reviewing , it was observed that the mine area recorded a higher level of SPM concentration. However, no instance of respiratory disease due to dust has come to notice. Hill facing the factory on the north-eastern side has lost most of its vegetation. No visible foliar injury on plants was observed, which might have been because of the low concentration of SO_2 in the ambient air.

Keywords: Sulphur Dioxide, Nitrogen Oxide,Suspended Particulate Matter,Ambient Air, Chlorophyll, Dust particles, Raw magnesite, Kilns and Photosynthesis

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Introduction

The main component of the Earth's atmosphere is air. It is the layer of gases that surrounds the planet. Air helps to protect life on Earth by absorbing harmful ultraviolet solar radiation and creating pressure which allows water to exist on the surface of the Earth. Moreover , it warms the surface through heat retention (greenhouse effect) and assists in diurnal temperature variation. A crucial constituent of Air is nitrogen (78%), followed by oxygen (21%), argon (0.9%) and 0.1 per cent of other gases. These other gases are known as trace gases that include greenhouse gases such as carbon dioxide, methane, water vapour and neon. An increase in the composition of these gases is directly proportional to a rise in Earth's temperature, also known as global warming.

The air pollution appeared from two sources: Point and Non point Sources.

Point Source

The dust generated during drilling, blasting, shoveling, transportation and unloading have increased suspended particulate matter (SPM).

Non-Point Source

The Dead Burnt Magnesite (DBM) plant which is established at a distance of about 8 km from the mines causes particulate and gaseous emission through two sources:

(i) Crushing and Grinding unit

(ii) A stack of vertical shaft kilns (2 Nos)

The Plant has made following provisions to control air pollution.

Crushing and Grinding Unit

To control the dust from crushing and grinding unit, the cyclone for the collection of coarser particles and filter bags for finer dust are provided. The dust-free gases are allowed to emit from a chimney provided for thepurpose.

Kilns

Each Kiln is equipped with two number of the cyclone before exhaust , which collects 90-95% of the dust.The composite process of mining involves drilling, blasting, crushing, grinding, loading, unloading and transporting, releasing a considerable amount of dust into the atmosphere. It was observed that top soil layer has become covered with a thin coating of magnesite dust generated from DBM plant and mine area which in the presence of moisture creates encrustation leading to anaerobic conditions and changes in soil's physical chemical properties and its consequent deterioration .Despite the measures taken by the plant to mitigate air pollution, the analysis of ambient air quality becomes necessary. The observations are given under the chemical analysis of ambient air.

Air Sampling

The location of sampling points selected for the evaluation of ambient air quality is shown in Fig. 1. The sampling frequency is given in the Tables 1, 2 and 3. All the samples were taken in the month of March. The wind direction was taken into account at the time of sampling. The prevalent wind direction was observed to be West to East. An eight hourly sampling was done with a view to determining the concentration of suspended particulate matter (SPM), SO_2 and NO_2.

Active air sampling was followed in which air is passed through a tube that is filled with a solid sorbent material. The sorbent material chemically absorbs the contaminant. A sampling pump is used to collectan air sample through this method.

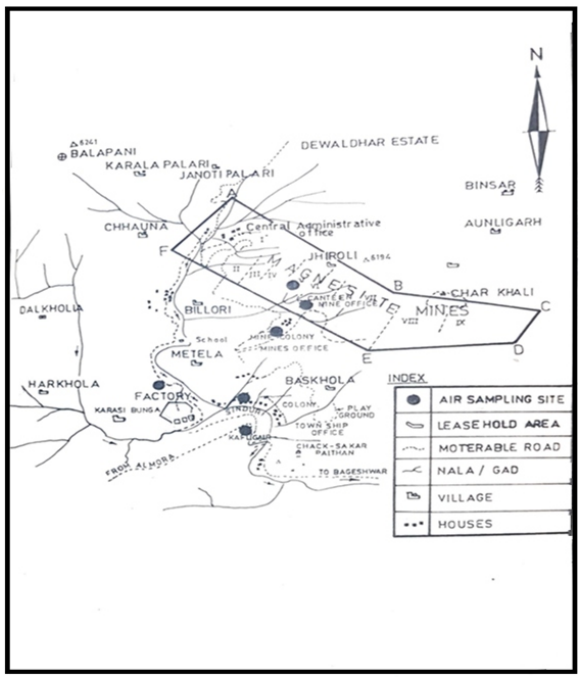

Fig. 1. Ambient Air Sampling Stations

Description of Sampling Sites

1. This point is at a distance of 300 meters from the plant near plant township.
2. This point is at a distance of 350 meters from the plant near the village Kafligair.
3. This point is at a distance of 250 meters from plant towards village Matela.
4. This point is near dressing yard (mines).
5. This point is near the Mines office.
6. This point is near Crusher.

Instruments and Methodology

(i) Suspended Particulate Matter (SPM)

Envirotech's APM 410 High Volume Sampler was used for measuring concentration of Suspended particulate matter in atmospheric air.Envirotech's APM 410High Volume Air Sampler is an improved version of an earlier model APM 400. It has been widely accepted as a standard instrument for measuring the concentration of Suspended Particulates.

This instrument consists of a heavy duty Blower which is used to suck atmospheric air through

the filter. The volumetric flow of air drawn through the filter is measured and the filter paper is weighed before and after sampling to determine the amount of dust deposited on it.The weight of suspended solids per unit volume of air can be calculated from these. The concentration of PM10 in the designated size range is calculated by dividing the weight gain of the filter by the volume of air sampled.

(ii) Sulphur Dioxide

The Sulphur Dioxide was determined through Modified West &Gaeke Method (IS 5182 Part 2 Method of Measurement of Air Pollution: Sulphur dioxide).

Sulphur dioxide is absorbed from air in a solution of potassium tetrachloromercurate (TCM). A dichlorosulphitomercurate complex which resists oxidation by the oxygen in the air, is formed. Once formed this complex is stable to strong oxidants such as ozone and oxides of nitrogen and therefore the absorber solution may be stored for some time prior to analysis. The complex is made to react with para rosaniline and methylsulphonic acid. The absorbance of the solution is measured by means of a spectrophotometer.

(iii) Nitrogen Oxides

The Nitrogen oxides (as Nitrogen dioxide) were determined through Modified Jacob &Hochheiser Method (IS 5182 Part 6 Methods for Measurement of Air Pollution: Oxides of nitrogen).

Ambient nitrogen dioxides(NO_2) are collected by bubbling air through a solution of sodium hydroxide to form a stable solution of Sodium nitrate. The concentration of nitrite ion (NO_2) produced during sampling was determined colorimetrically by reacting the exposed absorbing reagent with Phosphoric acid , Sulphanilamide, and N(1-naphthyl) ethylenediamine dihydrochloride (NEDA) and measuring the absorbance of the highly colouredazo-dye at 540 nm.

Chemical Analysis of Ambient Air

Table 1 Ambient Air Quality

S.No.	Sampling Site	Sespended Particulate Matter (SPM) g.m ^{3}			Mean g.m ^{3}
		A	B	C	
1.	I	88.50	80.00	78.50	82.33
2.	II	68.30	66.50	62.00	65.60
3.	III	61.40	59.00	54.50	58.30
4.	IV	98.46	NR	NR	98.46
5.	V	95.20	NR	NR	95.20
6.	VI	115.70 NR	NR	115.70	
	Mean	87.92	68.5	65.0	

A = 9.30 AM to 5.30 PM NR = Not Recorded
B = 5.30 PM to 2.30 AM
C = 2.30 AM to 9.30 AM

Table 2 Ambient Air Quality

S.No.	Sampling Site	Sulphur dioxide Concentration g.m ^{3}			Mean g.m ^{3}
		A	B	C	
1.	I	20.2	18.76	15.42	15.30
2.	II	14.6	11.8	9.82	12.07
3.	III	6.24	4.32	3.10	4.50
	Mean	13.68	11.62	9.44	10.62

A = 9.30 AM to 5.30 PM
B = 5.30 PM to 2.30 AM
C = 2.30 AM to 9.30 AM

Table 3 Ambient Air Quality

S.No.	Sampling Site	Nitrogen oxide Concentration g.m ^{3}			Mean g.m ^{3}
		A	B	C	
1.	I	15.06	13.96	12.82	13.94
2.	II	14.76	10.42	9.21	11.46
3.	III	9.72	6.25	6.05	7.34
	Mean	13.18	10.21	9.36	10.91

A = 9.30 AM to 5.30 PM
B = 5.30 PM to 2.30 AM
C = 2.30 AM to 9.30 AM

Table 4 Meteorological observations obtained during sampling period

S. No.	Direction of the Sampling Site from the Source		Distance from the source, meter	Wind Speed Km.h ^{1}	Wind direction from	Humidity , %
1	I	East	300 meter	3.0	W to E	54 %
2	II	SE	350 meter	3.4	W to E	52 %
3	III	NW	250 meter	3.6	W to E	51 %

Results

The results of ambient air quality with respect to SPM, SO2and NOx are given in Table 1,2 and 3. Meteriological observations obtained during the period are given Table 4.

The observed atmospheric concentration for pollutants namely SPM, SO2 and NOx were found to be well within the ambient air quality standards of the Central Pollution Control Board (CPCB) as given below. The mean SPM concentration around plant area was 68.74 -3. While the maximum SPM concentration (115.7 m ^{3}) was recorded from crusher at mine area, the mean maximum and minimum SPM concentration around plant area were obtained from township area (82.33 g. m ^{3}) and village Matela side (58.3g.m ^{3}) respectively.

The mean Sulphur dioxide concentration was observed as 10.62 m ^{3}, while the mean concentration of nitrogen dioxide was 10.91m^{-3}. Since this is the only industry in the area, so whatever airborne dust is created, it gets settled down fast and thus leaving very little amount suspended in the air column.

Table 5 : National Ambient Air Quality Standard, 2009 Central Pollution Control Board

Pollutant	Time Weighted Average	Concentration in Ambient Air	
		Industrial, Residential, Rural and other Areas	Ecologically Sensitive Area
Sulphur Dioxide	Annual	50	20
(SO_2) g / m^3	24 Hours	80	80
Nitrogen Dioxide	Annual	40	30
(NO_2) g / m^3	24 Hours	80	80
Particulate Matter	Annual	60	60
(Size less than 10 *m*) or PM10, g / m^3	24 Hours	100	100
Particular Matter	Annual	40	40
(Size less than 2.5 *m*) or PM 2.5, g / m^3	24 Hours	60	60

Depending upon the prevalent wind direction, atmospheric pollution in the area can be attributed to the emissions from the Dead Burnt Magnesite Plant. Besides, dust from mining area also results in the air pollution. The burning of raw magnesite in the shaft kilns causes emissions of SO_2, NO_2 and Suspended particulate matter, consisting of oxides of Mg, Si, Fe, Al and Ca, Fig. 2 indicates the effect of emissions from the plant on the vegetation of hills around the factory area and Fig. 3 indicates that the ambient air of village Matela is being affected by the emissions from the plant.

Fig. 2. The emissions from the plant has affected the vegetation on nearby hill

Fig.3.The village Matela is being engulfed by the emission from the plant.

Discussion

The values of SPM, SO_2 and NO_2 were 76-148 .m-3 (AV 105 m ^{3}) 2-23 and 10 m ^{3} (AV 4.4 m ^{3}) respectively at the mine and the plant during the month of May (Pichamuthu, 1993).

Out of the use of 200 tonnes of explosives in a mine, about 20 million m3dust is released into the atmosphere (Lodha *et al*., 1995).

Particles of dust and Carbon remain coated with toxic gases emanating from plant emissions and automobile exhaust. The SPM coat the lungs and cause respiratory infections, persistent cough and throat irritation also aggravate asthma (India Today, Dec'96). The dust-borne diseases depend upon the chemical composition of dust, size of particles, duration of exposure of workers and individual susceptibility. Though magnesite dust contains silica, this silica is not in uncombined form. No instance of silicosis has ever come to notice. To safeguard its workers against dust related health hazards, the company has provided dust masks. Besides, the raw Magnesite dust particles normally are of 0-50 mm size. The particles of health significance range between 0.2 to 10 microns (Bloor, 1961). The common incidence of Bronchitis among the hill people of the area appear not because of non-respirable dust but the wood fire inside their closed rooms where damp mud floor is used for sleeping is contributing to pulmonary diseases. Apart from this, hill people began smoking from their early childhood which also accelerates pulmonary diseases.

There is a concern over the increasing concentration of airborne pollutants in the atmosphere. The air pollutants not only cause physiological disorders in plants but also effect changes in the structure and function of the ecosystem through a variety of ways. The contaminants may be gaseous or particulate. Among the gaseous pollutants, oxides of sulphur, fluorides, ozone, nitrogen oxides and PAN (peroxyacetyl nitrate) are the major ones (wood,1968). Apart from gaseous pollutants, "Particulate matter" (organic and inorganic) is also released in the atmosphere from various sources viz, household, commercial, industrial and power. Important particulates are dust of iron oxides, fly ash, catalyst dust ash, chemical coke dust, lime dust and cement dust.

In Germany, it was recognized that smoke contained both solid particulates and gases. Stockhardt (1850, 1871) was the first to recognize the relation between the sulphur content of coal and the damage caused to plants by SO_2 produced from burning coal. Wislicenus (1914) observed that gases were more damaging to plants than particulates. Stoklasa (1923) suggested that SO_2 might reduce plant growth without causing visible foliar injury. The absorption of SO_2 into leaves is regulated by stomata (Wells, 1917). Morphological observations reveal that leaf morphology plays a major role in capturing dust from the atmosphere (Le Blanc and Rao, 1973, Jacobson, 1980, Yunus *et al*., 1985, Pyatt and Haywood, 1989).

Plants exposed to low concentrations of pollutants may manifest a reduction in growth and yield without any visible injury symptoms, though subtle changes in biochemical and physiological functions may occur. According to Rao and Rao (1989), sulphur dioxide is phytotoxic in a concentration above 0.1-0.2 ppm. Below 0.4 ppm, it tends to be oxidized in the cells as rapidly as it is absorbed, but interferences with functions such as photosynthesis are slight; the chronic injury is generally exhibited with these small concentrations. Above this, acute injury characterised by the killing of marginal orinterveinal areas at the leaf occurs more frequently. Sometimes temporary interference with photosynthesis or invisible injury can occur.

Clhloorophyll-a is degraded to phaeophytin by SO2 through replacement of Mg^{2+} ions from chlorophyll molecules but chlorophyll b degradation leads to the formation of chloropliyllide b as SO_2 removes phytol group of the chlorophyll b molecules (Rao and LeBlanc, 1965). SO_2 Causes local acidity which splits Mg^{2+} from chlorophyll, converting it into phaeophiytin. This causes chlorosis.

SO_2 destroys chlorophyll content in plant and replace Mg+ of chlorophyll molecule by two atoms of hydrogen forming Phaeophytin and changing of light spectrum charateristics of the chlorophyll molecule and reducing the photosynthetic activity. (Voet and Voet,1990)

Both NO and NO_2 can alter apparent photosynthesis and respiration. Hill and Bennett (1970) found that apparent photosynthesis in alfalfa (Medicago sativa) and oat (Avena sativa) plants were reduced by two hour exposure to either NO (0.74 mg/m^3) or NO_2 (1.13 mg/m^3), but in both the species the rates of photosynthesis quickly returned to pre-exposure levels, When alfalfa and oats were exposed to equal concentrations of the two gasses, apparent photosynthesis in both the species was inhibited by NO_2 than by NO, but NO caused greater initial inhibition. Apparent photosynthesis in tomato (Lycopersiconesculentum) was reduced both by NO (0.65 mg. m-3) and by NO_2 (0.9 mg.m-3), and prolonged exposure to either pollutant ultimately reduced growth (Capron and Mansfield, 1976). Oxides of nitrogen are less phytotoxic than SO_2 or ozone (O_2) and concentrations of NO_2 that presently occur in ambient air hardly pose any threat to crop productivity. The effect of NO_2 on crop growth and yield are minimal in comparison with the effect of NO_2 in combination with SO_2 and O_2 (Amundson and Maclean, 1982).

Mitigation measures

Two numbers of cyclones are provided with each kiln before exhaust which collects 90-95 % of the dust. The balance dust along with the flue gases are diverted to fabric filter dust collector unit. The dust-free flue gases are emitted into the atmosphere through 30 m high Chimney.

In order to monitor the level of SPM, the company has established air sampling stations at View point, Dressing Yard Colony, mines office and near crusher. The company has provided for 7 KL capacity tanker for sprinkling water on the roads in the mine area.

The dust particles are produced during every industrial activity whenever any material undergoes disintegration. The composite process of mining consisting of drilling, blasting, shoveling, crushing, grinding, loading, transportation and unloading release a considerable amount of dust into the atmosphere. The gaseous pollutants emitting through the use of diesel equipment like shovel, dumpers, dozers, trucks and compressors also increase the level of SPM. The vehicle blown dust, road construction also increase the level of SPM. The vehicle blown dust, road construction activities in the area, and smoke from the burning of fuelwood in houses and the forest fire is also contributing to the SPM. Besides, hilly terrain and brisk wind velocity also help in the heavy dispersal of dust in the area.

Conclusion

The composite process of mining involving drilling, blasting, crushing, grinding, loading, unloading and transportation release a considerable amount of dust in the atmosphere. The level of SPM concentration was recorded to be higher in the mine area. However, no instance of respiratory disease on account of dust has come to notice. As a result of toxic emissions from the factory, though within the ambient air quality standards prescribed by the Central Pollution Control Board, hill facing the factory on the north-eastern side has lost most of its vegetation. But elsewhere no visible foliar injury on plants has been observed which may be because of low concentration of SO_2 in the ambient air.

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RESEARCH ARTICLE

A Review: Types of Bioreactors and its application for Sustainable Environment

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Abstract

Biotechnology is not just the sum of microbiology, genetics, biochemistry, engineering, etc.; no, it is an integration of these disciplines, and that involves much more than simple addition. Integration and application are key words found in most definitions of biotechnology. In particular, the integration of biological and engineering principles is essential when designing bioreactors. The bioreactor should be designed to meet the specific biological and technological requirements of the process. Of course, the quality and price of the product are decisive for commercial implementation. Thus, the goal of bioreactor design can be defined as "minimizing the cost of the product in question while maintaining the desired quality within biological and technological constraints." This does not a priori mean that minimizing the cost of the bioreactor also means minimizing the cost of the integral process. Bioreactor systems play an important role in tissue engineering because they enable reproducible and controlled changes in specific environmental factors. They can provide the technical means to conduct controlled studies aimed at understanding specific biological, chemical or physical effects. In addition, bioreactors enable the safe and reproducible production of tissue constructs. For later clinical applications, the bioreactor system should be an advantageous method in terms of low risk of contamination, easy handling and scalability.

Keywords: Bioreactor, Enzymes, Microorganism, Culture

Introduction

The primary unit of industrial biochemical transformation is the bioreactor, where treated materials are transformed by the activity of living cells or biological elements like enzymes. In bioreactors, raw materials are converted into biochemical products and/or less undesired by-products by cells or cell-free enzymes. These reactors are commonly cylindrical, ranging in size from a litre to some cube meters, but differs depending on the design and the operation mode in industrial bioprocesses. Although the bioreactor may be simple or highly instrumental, its ability to produce the desired product or results is important to consider. The bioreactor is built and run to create the environment necessary for the production of the products that scientists, bakers, or winemakers have chosen. It serves as the brain of numerous biotechnological systems that are employed in industrial, medicinal, agricultural, and environmental applications (Schaechterand Lederberg, 2004). Due to the production of products in these devices, which are the core of the bioprocesses, all bioreactors are of utmost importance (Cinar *et al*., 2003). The bioreactor may occasionally be used to produce biomass (such as single cell proteins, Baker's yeast, animal cells, or microalgae),

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metabolites (such as organic acids, ethanol, antibiotics, aromatic compounds, and pigments), substrates (such as steroids), or even active cell molecules (e.g. enzymes). Tissue culture systems are those that are based on the cultivation of mammalian or plant cells, while "microbial" reactors are those that are based on the distributed non-tissue culture of microorganisms (bacteria, yeast, fungi) (bioreactor, fermenter). No living cells are used in the enzyme reactors to transform the substrate. These reactors frequently use immobilised enzymes, which are biocatalysts that are attached externally or internally to solid supports to allow for repeated usage and reduce enzyme consumption (Bhattacharyya *et al*., 2008).

A bioreactor consists of a complex system of pipes, fittings, wires, and sensors; it is exposed to operational problems. With the aid of on-line monitoring and diagnosis tools, it is now possible to detect many things that can go wrong during the process (Cinar *et al.,* 2003).

The bioreactor has origin in early history, before 500 B.C. the Babylonians still produce beer in tanks which had the function of a bioreactor. Wine was produced in wineskins, which were carefully selected for their ability to produce a beverage that met the approval of the king and other members of his sensory analysis. Early recorded history shows that some understood the importance of the components and the environmental or operating conditions of the reactor. This allowed leavened bread and cheese to be produced Egypt more than 3000 years ago (Schaechter and Lederberg, 2004).

Bioreactor operation mode is classified in: batch processes, bed-batch and continuous processes. Normally these operations mode are used in submerged or liquid fermentations or during cell culture such as tissue culture or algae growth. Batch processes has increased significantly nowadays and are extensively used to produce specialty biomolecules for uses in chemical, biotechnological, pharmaceutical industries.

The production of these high value-added bioproducts contributes to a significant and growing portion of the revenue and earnings of bioprocess industries (Cinar *et al*., 2003). Batch processes refer to a partially closed system in which most of the materials required are loaded onto the bioreactor aseptically and are removed at the end of the operation. In a batch bioprocess, the only material added and removed during the course of operation is air/gas exchange, antifoam and pH controlling agents. Most modern bioprocesses incorporate adjustments to the medium to control conditions and to supply nutrients and compounds that promote biosynthesis of the desired product (Cinaret al., 2003).

Bioreactors for fed-batch processes represent an important class of bioprocesses, mainly in the food industry and in the pharmaceutical industry but also e.g. for biopolymer applications (PHB). One of the key issues in the operation of fed-batch reactors is to optimize the production of a synthesis product such as enzymes and penicillin or even biomass (Dochain, 2008). In fed-batch or also called semi-continuous bioreactor characterize by the feeding of sterile substrate, the absence of outflow from the fermenter and the increase in volume (accumulation of total mass) in the bioreactor. It can be used to demonstrate the important characteristics of quasi-ready state, linear growth, and use of alternative feed strategies (Dunn, 2003). Besides substrate, required nutrients also are added continuously or intermittently to the initial medium after the start of cultivation or from the point halfway through the batch process. Fed-batch processes have been utilized to avoid utilizing substrates that inhibit growth rate if present at high concentration, to overcome catabolic repression, to demand less initial biomass, to overcome the problem of contamination, and

to avoid mutation and plasmid instability found in continuous culture (Nag, 2008).

In continuous culture, fresh medium is added into the batch system at the exponential phase of the microbial growth with a corresponding withdrawal of the medium containing the product. The continuous cultivation gives a near-balanced growth, with little fluctuation of the nutrients, metabolites, cell numbers or biomass (Binod *et al*, 2008).

Differ from submerged fermentation, solid-state fermentation (SSF) has been defined as the fermentation process which involves solid matrix and is carried out in absence or near absence of freewater; however, the substrate must possess enough moisture to support growth and metabolism of the microorganism. The operation mode of solid state fermentation most used industrially is the batch system. Commonly used SSF bioreactors can be divided into four types based on type of aeration or the mixed system employed. These are tray, packedbed, horizontal drums and fluidized bed having their own advantages and disadvantages, which promoted the necessity to develop novel bioreactors with better design (Singhania *et al*, 2009). This process recycles agro-industrial residues without economic fate for many different applications in bioprocesses such as enrichment, biological detoxification, production of biomolecules such as enzyme, organic acids, food aroma compounds, biopesticides, mushrooms, pigments, xanthan gum, vegetable hormones (Soccoland Vandenberghe, 2003) and may be used different types of bioreactors for lead the solid state fermentation.

Batch operation systems can be applied for all types of bioreactors. However, fed-batch and continuous operation systems must be analysed according to the different models of bioreactors and the process itself. These operations present some advantages, compared to the batch systems, although they need some investments and rigorous instrumentation and control.

Different Types Of Bioreactors

Stirred Tank Reactors - STR

Bioreactors designed for the most efficient expression of the biological properties of the living cells must achieve optimal interactions between the cells and the culture media. In a closely controlled environment they have to provide efficient means of mixing, mass and heat transfer between the different phases. (Engasser, 1988). Current reactor technologies, new types of bioreactors are constantly being developed in order to optimize and improve productivities.

Because of its versatility and flexibility the mechanically stirred tank reactors (STR) remains the mainstay for industry. According to Najafpour (2008), there are three main types of fermenters that are used in industrial scale:

1- Non-mixed and non-aerated systems: approximately 70%
2- Non-mixed and aerated systems: approximately 10%
3- Mixed and aerated systems: approximately 20%.

Non-aerated and non-mixed tanks are used in the production of traditional products such as wine, beer and cheese. The major part of the new products are obtained from the cultivation of microorganisms, which is carried out in mixed and aerated tanks (Najafpour, 2008).

The main hole of the bioreactor is to provide an adequate and controlled environment for cell growth and product synthesis. In this way, there are several factors that must be considered in the construction of bioreactor. Among them sterility, aeration and mixing systems (when necessary), temperature and pH control, geometry, low energy consumption and adequate size and material (Stanbury, 1995).

The most important bioreactor for industrial applications is the conventional STR due to its low operation costs. The size of the tanks may vary between some dm^3 till hundreds of m^3. Laboratory-scale tanks with a volume of

maximum 20 liters are made of glass. For higher volumes, they are generally made of staining steel. Different materials and their combinations can be used for their manufacture.

The relation between high and diameter may vary between 2:1 till 6:1, depending on the heat to be removed (Najafpour, 2008). The ration 1:1 is probably the most economic because it presents a lower superficial area and, consequently, it needs less material. However, when the aeration is required, the aeration rate must be higher to promote a higher contact between the air bubbles and the liquid, but also a higher hydrostatic pressure on the top of fermenter (Doran, 1995).

In the bioreactor, the homogeneity and bubble dispersion is achieved by mechanical agitation, which requires relatively high energy consumption per unit of volume (Doran, 1995). There is a great variety of impellers that are described, which produce different flow patterns in the tank.

Generally, 70-80% of the total volume of the STR is filled with liquid. In this case, there is a headspace for gas exhaustion and foam formation, which can be controlled by a foam breaker.

STRs are used for free and immobilized cells and enzymes. The sensibility of the biocatalysers must be studied and analysed in each case (Doran, 1995).

Continuous Stirred Tank Reactors - CSTR

The CSTR are defined as STR that works in a continuous operation including feeding and remove of mass and energy. An ideal CSTR can be modelled considering a perfect mixing, without temperature, concentration, fluid properties and reaction rate variations.

It means that inlet and outlet streams have the same properties of the bioreactor medium. All properties of the CSTR are determined exactly as for the batch bioreactor. In this case, the instrumentation and control systems are different and more sophisticated (Laska & Cooney, 1999). There are two types of CSTR operation strategies. One of them is the Chemostat that is used for cell culture in which all nutrients are added in excess and the liquid volume is kept constant by setting the inlet and outlet flow rates equal. The second one is the Turbidostate where the cell concentration is maintained constant by the monitoring of the culture optical density and the liquid volume is kept constant by setting the outlet flow rate equal to the inlet flow rate. CSTRs can be used in series with more than one bioreactor with different conditions in each one.

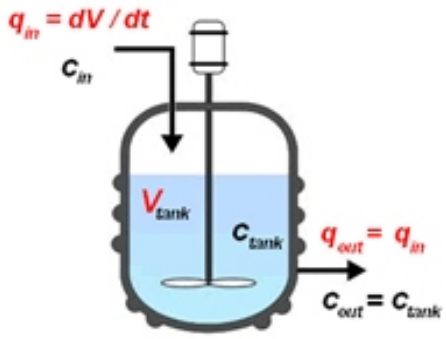

Fig. 1 Continuous Stirred Tank Reactors

Bubble Column Bioreactors

Bubble column reactors (BCRs) are pneumatic mixed reactors, which were developed for sensitive cells culture such as filamentous fungus cells, mammalian and plant cells.

A bubble column reactor (BCR) is basically a cylindrical vessel with a gas distributor at the bottom. The gas is sparged in the form of bubbles into either a liquid phase or a liquid–solid suspension. These reactors are generally referred to as slurry bubble column reactors when a solid phase exists. Bubble columns are intensively utilized as multiphase contactors and reactors in chemical, petrochemical, biochemical and metallurgical industries (Degaleesan, 2001; Kantarcia *et al*, 2005).

BCRs usually consist of a cylinder with a ratio high: diameter of 2:1 or even 3:1, differently to STRs, in order to allow a better time of contact of the air and the liquid. For some applications it is possible to find the ratio high: diameter of 6:1. On the top of the BCRs the diameter of the cylinder is larger to facilitate the liberation of bubbles and foam break. The aeration is promoted with compressed air through some spargers that are installed in the bottom of the tank. There are no other internal components.

Gas sparger type is an important parameter that can alter bubble characteristics which in turn affects gas holdup values and thus many other parameters characterizing bubble columns. The sparger used definitely determines the bubble sizes observed in the column. Small orifice diameter plates enable the formation of smaller sized bubbles. Some common gas sparger types that are used in literature studies are perforated plate, porous plate, membrane, ring type distributors and arm spargers.

Gas holdup is a dimensionless key parameter for design purposes that characterizes transport phenomena of bubble column systems (Luo *et al*, 1999). It is basically defined as the volume fraction of gas phase occupied by the gas bubbles. All studies examine gas holdup because it plays an important role in design and analysis of bubble columns (Kantarci*et al.*, 2005).

Some important applications of bubble columns are the production of industrially valuable products such as enzymes, proteins, antibiotics etc.

Although the construction of bubble columns is simple, accurate and successful design and scale-up require an improved understanding of multiphase fluid dynamics and its influences (Kantarci *et al.*, 2005). The most important parameters in this type of bioreactor are the bubble ascending speed, the residence time, the "hold up", the interfacial area and the mass transfer. Hold up is the proportion of liquid that is occupied by the gas or the bubble volume in relation to the liquid.

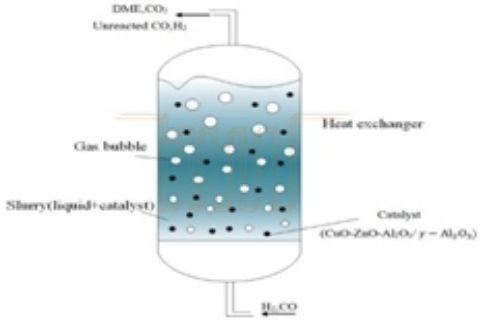

Fig. 2 Bubble Column Bioreactors

Air Lift Bioreactors

Airlift bioreactors (ARL) are a variation of the BCRs. The main difference is a central tube or other components (channels) that are responsible for an efficient mixing and recirculation of the fluid. This fact reduces the coalescence of bubbles, which circulate through the reactor, and equalize the shear stress that is provoked by the mixing. The term Airlift is linked to the characteristics of pneumatic contact of the gas-liquid or gas-liquid-solid defined by the circulation of the fluids in a cyclic pattern (Flickinger & Drew, 1999).

There are two main basic configurations of the ALRs: External loop reactors and internal loop reactors. In the first one, the circulation of the fluids follows distinct channels; in the second one there is only a barrier strategically positioned in a vessel, which creates some channels for the circulation or concentric tubes that creates a central and a peripheric channel (Flickinger and Drew, 1999).

There are some different structures of external loop reactors and internal loop reactors. These configurations can be re-worked with the development of new possibilities for the amelioration of the fluid dynamic and a better liberation of the gas in the liquid according to the different processes.

For example, in the internal loop with concentric tubes, depending on the number and position of spargers, the ascendent gas

flux can be produced at both the central or peripheric part of the bioreactor.

Scale-up studies of ARLs pass through the same analysis made for BCRs, where the superficial gas velocity, holdup and dynamic of the fluids must be analysed.

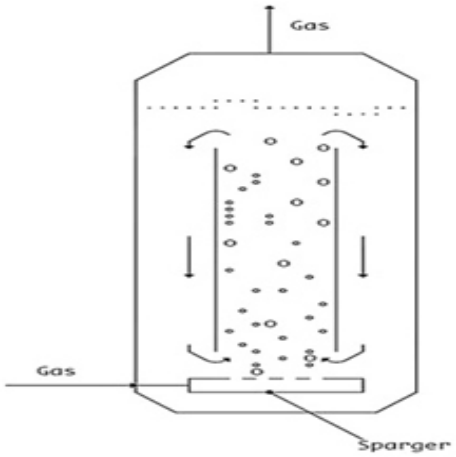

Fig. 3 Air Lift Bioreactors

Packed Bed Bioreactors

The Packed Bed Bioreactors (PBRs) typically consist of a packed-bed that supports the cells on or within carriers and a reservoir that is used to re-circulate the oxygenated nutrient medium through the bed. Two major configurations are possible, with the packed-bed compartment located either external to, or within, the reservoir of the medium (Wang *et al.*, 1992 a, b). A frequent approach in developing PBRs is to first use a small-scale model bed to identify the optimal packing matrix for the cell line of interest. An optimal matrix is one that provides the requisite combination of cell attachment, proliferation and productivity. This matrix is then used to optimize the operational parameters (e.g., packed-bed height and volume, medium perfusion rate, etc.) of the PBR through perfusion experiments that are generally performed at laboratory-scale (Meuwly*et al.*, 2007).

There has been an increasing trend in identifying support materials that were compatible with different types of cells (microorganism's cells and mammalian cells). Higher internal porosities ranging from 0.80 to 0.95 were reached with the next generation of packing materials such as disks made of non-woven polyester and polypropylene screen, ceramic spheres and other shapes, glass fibers (Perry and Wang, 1989; Chiou*et al.*, 1991), polyurethane and polyvinyl foams or resins (Meuwly*et al.*, 2007).

PBRs can provide extremely high productivity within a compact size is the. PBRs have been used widely for perfusion culture of immobilized mammalian cells. Many authors presented the potential of the use of PBRs as "artificial organs" (Allen *et al.*, 2001) in biomedical applications. A relatively well-known example of such application is the bioartificial liver device (BAL) (Allen and Bhatia, 2002).

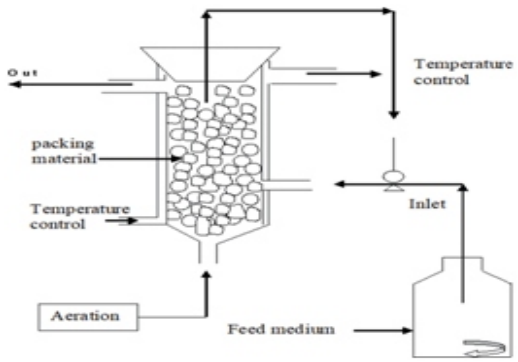

Fig. 4 Packed Bed Bioreactors

Fluidized Bed Reactors

Fermenters for fluidized bed (FB) operation are tall columns, where the ratio of height to diameter (aspect ratio) is typically greater than 10:1. Dempsey (1994) reported that fermenters with an aspect ratio of either 20:1 or 40:1 have been designed and operated. The column diameter should ideally be at least 50-times the particle diameter; but in the laboratory it is often necessary to compromise between this ideal ratio and the need to minimize the volume of the fermenter. Typically, lab-scale fermenters have a ratio between 25:1 and 40:1. The fluidizing medium can be gas, liquid, or a mixture of the two; with

the flow being upwards for fluidization of particles denser than the fluid, or downwards for particles of lower density, in the case of the fluidized bed fermentation (FBF)s, the fluidizing medium is usually the broth; and for biological fluidized bed (BFBs), the wastewater; with the liquid flowing up through the bed.

As well as applications in wastewater treatment, FB technology can also be applied to pure culture fermentations for the production of microbial metabolites or biomass.

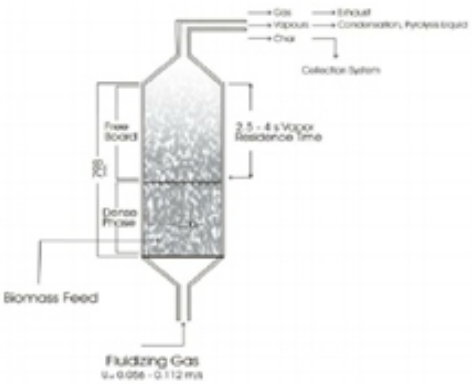

Fig. 5 Fluidized Bed Reactors

Membrane Bioreactors

Since 1990, membrane bioreactors (MBR) are been used. They are mainly composed by installations that are constructed in external configuration, in which case the membrane modules are outside the bioreactor and biomass is re-circulated through a filtration loop. After the mid-1990s, with the development of submerged MBRsystem, MBR applications in many areas extended widely (Meng *et al*, 2009).

Membrane bioreactor (MBR) technology is advancing rapidly around the world both in research and commercial applications. Several generations of MBR systems have evolved. Up to this date, MBR systems have mostly been used to treat industrial wastewater, domestic wastewater and specific municipal wastewater, where a small footprint, water reuse, or stringent discharge standards were required. It is expected, however, that MBRsystems will increase in capacity and broaden in application area due to future, more stringent regulations and water reuse initiatives (Cicek 2003; Visvanathan *et al.*, 2000; Yang *et al.*, 2006; Meng *et al.*, 2009).

In the water and effluent treatment context, an MBR comprises a conventional activated sludge process coupled with membrane separation to retain the biomass. Since the effective pore size is generally below 0.1 μm, the MBR effectively produces a clarified and substantially disinfected effluent. In addition, it concentrates up the biomass and, in doing so, reduces the necessary tank size and also increases the efficiency of the biotreatment process (Santos *et al.*, 2011). MBRs allow high concentrations of mixed liquor suspended solids (MLSS) and low production of excess sludge, enable high removal efficiency of biological oxygen demand (BOD) and chemical oxygen demand (COD), and water reclamation. However, membrane fouling is a major obstacle to the wide application of MBRs. Additionally, large-scale use of MBRs in wastewater treatment will require a significant decrease in price of the membranes (Meng *et al.*, 2009).

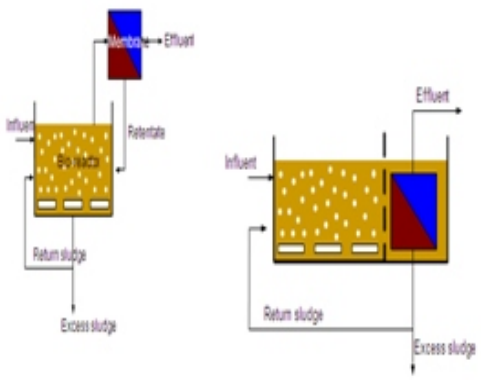

Fig. 6 Membrane Bioreactors

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RESEARCH ARTICLE

Development of Protocol for Total RNA Isolation from *Selaginella bryopteris*

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Abstract

Extraction of quality RNA from xerophytic plants like *Selaginella bryopteris* for molecular biology applications is problematical due to the presence of many secondary metabolites. The existing protocols are not suitable to yield quality RNA in adequate quantity from fronds of *Selaginella bryopteris*. Therefore, most common method i.e. CTAB-based protocol was optimized and partially modified to solve the purpose. This modified protocol gave high quantity (500.00 ìg/g fresh weight of leaf tissue) of quality RNA based on qualitative and quantitative analysis by spectrophotometer and electrophoresis respectively.

Keywords: *Selaginella bryopteris*, RNA isolation, Xerophytic plant, Electrophoresis

Introduction

Selaginella bryopteris, also known as Sanjeevani, is an herbaceous xerophytic pteridophyte that normally grows in a rainy season like a lush green mat on hills and gradually dries up hay like thereafter, particularly during water stressed condition. This plant has resurrection capability, survives intense drought and rejuvenate when soaked in water even after being uprooted and dried. Therefore, drought resistance gene of this plant may be used in molecular breeding for the development of drought tolerant/ resistant crop line. For most of the gene identification and expression studies, isolation of total RNA is prerequisites. But this herb is rich in secondary metabolites, such as alkaloids, phenol (Flavonoids, tannins, saponins), and terpenoids (Triterpene, and steroid). Therefore, gene expression analysis is not easy due to high accumulation of secondary metabolites and other compounds in this crop that are capable to degrade RNA. Most common developed protocol and /or commercially available reagent-based methods (TRIzol®, Invitrogen, USA) or RNA isolation kits (RNeasy®, Qiagen, Germany) are not suitable in case of *Selaginella bryopteris* because of the reduced RNA yield and quality. Therefore, we modified and optimized protocols[4], to develop suitable isolation protocol from *Selaginella bryopteris* which is reported to be applicable for the isolation of total RNA from the crops rich in polyphenols and polysaccharides. This optimization and modification were only poised at removal of polyphenolics, polysaccharides and other secondary metabolites with the help of insoluble, inert material Polyvinyl polypyrrolidone (PVPP) to give rise optimum yield of RNA leading to downstream application.

Material and Methods

Selaginella bropteris was collected from the Girihinda hills located in Sheikpura district of Bihar, India.

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Fronds of this plant was used for RNA isolation after washing with sterile Di-Ethyl Pyro-Carbon (DEPC) treated water, blotted on tissue paper, quickly frozen in liquid nitrogen (LN) before isolation.

Solutions and Reagent

Glassware was baked overnight at 180°C before being used. Glassware including Mortar and pestle were treated with 0.1% (v/v) DEPC treated water and autoclaved before use. While Plastic wares used in this process was totally fresh and sterilized. Electrophoretic apparatus was also treated with 5% hydrogen peroxide / 80% ethanol for half an hour and air dried. Even solutions to be used in the RNA extractions, excluding which contain Tris, were also treated with 0.1% (v/v) DEPC. The purpose of DEPC is to inactivate the RNAse which may degrade the RNA and reduce the quality of RNA for downstream application. Gloves of molecular biology grade were used throughout the experiments to avoid potential contamination. Following reagents and chemical were used in this protocol of RNA isolation.

Table 1: Reagents and Chemicals used in this developed protocol for the isolation of DNA

S.No.	Name of Reagents/ Chemical	Strength
A.	Polyvinylpolypyrrolidone (PVPP)	4% (w/v)
B.	Phenol (water saturated): Chloroform: Iso-amyl alcohol	25:24:1, v/v/v
C.	Chloroform: Isoamyl alcohol	24:1, v/v
D.	LiCl	Both 6 M and 2 M
E.	Sodium Acetate (NaOAc, adjusted to pH 5.2)	3 M
F.	Ice cold Ethanol	Both 70% (v/v) and Absolute
G.	DEPC Treated sterile water	0.1% DEPC
H.	Extraction buffer{Tris-HCl: Sodium Chloride, Ethylene Diamine Tetra Acetic acid: Spermidine-Magnesium Chloride: Cetyl-Tri-Methyl Ammonium Bromide (CTAB): *â*-mercaptoenathol: Poly Vinyl Polypyrrolidone (PVPP)}	0.1 M (pH 7.0): 1 M: 10 mM (pH 7.5): 0.5 g/L: 5 mM: 2% (w/v): 3% (v/v): 3% (w/v).

Isolation of RNA

Frozen fronds of *Selaginella bryopteris*(1g) was ground to fine powder in a mortar and pestle with the help of Liquid Nitrogen (LN) and PVPP (3%) and was homogenized in 10 ml of extraction buffer consisting of 400 µl of â-Mercaptoethanol (3% v/v). 10 ml of Phenol (Water saturated): Chloroform: Isoamyl alcohol (25:24:1, v/v/v) was added and mixed completely. The suspension was milky white in color. Thereafter, this mixture was transferred into a 50 ml centrifuge tube for centrifugation at 15,000 rpm (Rotation per minute) for 15 min. The upper colorless aqueous phase rich in nucleic acids, was transferred to a fresh oak ridge centrifuge tube and equal volume of chloroform: Isoamyl alcohol (24:1, v/v) was added, and mixed by inverting the tube for about 20 times. The mixture was further centrifuged at 15,000 rpm for 10 min at 4°C, and the aqueous phase was transferred to another fresh oak ridge centrifuge tube. 6 M Lithium chloride (LiCl) was added to a final concentration of 3 M and incubated at (-80°C) for 5 h or at (-20°C) for 10 h. After incubation the solution was centrifuged at 15,000 rpm for 30 min at 4°C which resulted in the precipitation of RNA. The precipitate was again suspended in 4 to 6 ml of 2M LiCl and centrifuged at 15000 rpm for 20 min at 4°C. RNA pellet was washed with 70% ice cold ethanol after discarding of supernatant. The pellet was then air dried to make it alcohol free. This alcohol-free pellet was dissolved in 250-500 µl of DEPC treated autoclaved water. 2 µl of the total RNA was loaded on formaldehyde denaturing agarose gel (1%, w/v) to check the quality of RNA.

Isolation Of High-Quality RNA

The isolated RNA still contains very few quantities of DNA due to almost similar physiochemical nature of RNA with DNA. Therefore, DNase (2 U/µg DNA) was added with 10X DNase buffer to a final concentration of 1X in the RNA solution. Thereafter, it was incubated at 37°C for 1 hour.

An equal volume of Chloroform: Isoamyl alcohol (24:1, v/v) was added in the solution. The resulting mixture mixed was mixed thoroughly by inverting the tube about 20 times. The mixed solution was centrifuged at 10,000 rpm for 10 min at 4°C. The upper aqueous phase was transferred diligently to a fresh 1.5 ml RNase free and sterilized Eppendorf tube. 1 volume of 3 M Sodium acetate (pH 5.2) and 2.5 volumes of absolute ethanol were added, mixed by inversion and incubated over night at -20°C. The content was further centrifuged at 12,000 rpm for 30 min at 4°C. The pellet formed after centrifugation was collected and washed twice with 70% (v/v) alcohol and air dried to make it alcohol free. The RNA pellet was subsequently dissolved in autoclaved and distilled water (100 to 250 µl) for qualitative and quantitative estimation.

Estimation of Quantity and Quality of RNA

The estimation of quality and quantity of RNA is a prerequisite for further downstream application. The quality of total isolated RNA was assessed using UV-VIS spectrophotometer by observing the ratio of absorbance / optical density at 260/280 and 260/230 nm. RNA quantity was determined by standard formula, 1 O.D. at 260 nm = 40 ng of RNA (Sambrook *et al*., 1989) Integrity and intactness of the RNA samples were examined in a denaturing agarose (1%w/v)gel, stained with ethidium bromide (EtBr), and visualized under UV transilluminator.

Results and Discussion

The fronds of *Selaginella bryopteris* is rich in flavonoids and other secondary metabolites that gets oxidized rapidly while sample grinding (Chan *et al*., 2009, Memon *et. al.,* 2010). Based on the species of plants and chemical composition of tissue many protocols are available for RNA isolation (Sharma, Gill PK and Singh 2003; Gasi Hernandez and Korban 2004). These methods do not hold good for all tissues and plant species. Above mentioned methods fail to isolate quality RNA in adequate quantities from plants which are rich in secondary metabolites. The secondary metabolites probably interact with other molecules and interferes the process of isolation RNA by solvent extraction. The complications have been seen while isolation of RNA from plants of same and different species as well. Some protocols even failed to yield quality RNA in sufficient quantities in other crops (Srivastava 2012; Morante-Carriel 2014). Therefore, we modified the existing protocol, which was applicable in the isolation of RNA from similar nature of tissue to a great extent (Table1). This partially modified protocol gave rise quality RNA in adequate quantities from the pteridophyte *Selaginella bryopteris*. RNase inhibitors, chelator (EDTA), and protein denaturants (â-mercaptoethanol) were the main components of the reaction mixture. This modified protocol yielded a white, water soluble RNA precipitate from fronds of *Selaginella bryopteris*.

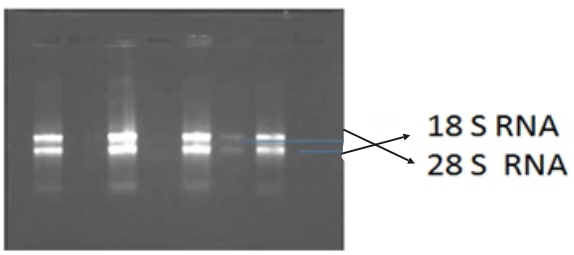

Figure1: Band of 28S and 18S rRNA while electrophoresis under denaturing agarose gel stained with ethidium bromide.

There were clear distinct bands of 28S and 18S rRNA when there was electrophoresis under denaturing agarose gel stained with ethidium bromide (EtBr) (Figure 1). Total RNA harvested was 500.00 ± 2.00 ìg per gram of fronds and the ratios of 260/280 and 260/230 were 2.00 ± 0.02 and 2.05 ± 0.02, respectively. These values suggest very low contamination of interfering compounds (Table 2).

Table 2. Quantification of total RNA (Average of 3 replications ± Standard Error).

Species	Tissue type	Concentration (μg/g Fresh Weight)	Absorbance ratio	
			(A260/A280)	(A260/A230)
S. bryopteris	Fronds	500.00 ± 2.00	2.00 ± 0.02	2.05 ± 0.02

Therefore, we can believe firmly that this modified protocol could be useful for isolation of RNA from other xerophytic plants too which have resurrection capability. However, using this protocol, we could isolate quality RNA in sufficient quantities from other leaf tissues which has low moisture content.

Conclusion

The developed protocol mentioned above iseasy, economic and feasible for isolating total RNA from tissues with high levels of polyphenolics and secondary metabolites. The protocol may be useful to isolate intact RNA from plant tissues exposed to water stress, without any side effect on downstream applications.

Acknowledgments

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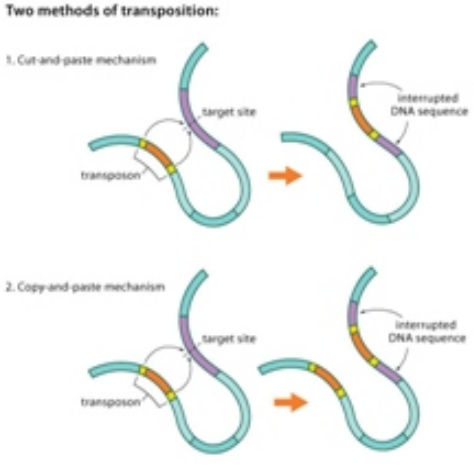
BIOTECH TODAY NEWS

Paving the way to bananas resistant to Fusarium oxysporum TR4

The Plant Breeding and Genetics Section (PGS) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture established a mutation breeding programme for the development of Cavendish bananas resistant to the soil-borne fungus Fusarium oxysporum, a destructive pathogen that damages the roots of banana plants. In the 1990s, a new strain of the fungus appeared in Southeast Asia called TR4 which caused enormous damage to banana cultivations. TR4 poses considerable risks not only to the banana industry but also to the food security of populations in many producing countries. Spores of the fungus can remain active in the soil for more than ten years and the disease can easily spread among other plantations. Recently TR4 was reported in Latin America. In the mutation breeding programme, a large mutant population of the Cavendish banana cultivar Grande Naine was produced and screened for resistance to TR4. Eight banana mutants showed no disease symptoms after artificial inoculation, paving the way to the development of TR4 resistant bananas.

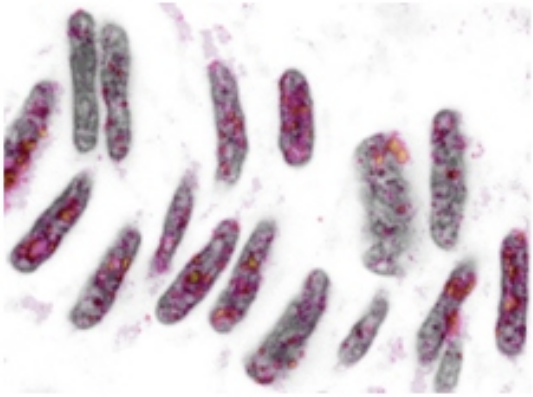
Tiny jumping genes fingered as culprit in rise of antibiotic resistance

Biomedical engineers at Duke University believe they have discovered the physical mechanism that causes high doses of antibiotics to promote the spread of antibiotic resistance between bacteria. The culprit, they say, is an overabundance of "jumping genes," called transposons, that carry the genetic instructions for resistance from the cell's source code to plasmids that shuttle between cells.The results appeared online March 28 in the journal *Nature Ecology & Evolution*."There's a lot of evidence that suggests human pathogens likely pick up antibiotic resistance from other species living in the natural environment," said Lingchong You, professor of biomedical engineering at Duke. "Intuitively, it makes sense that high levels of antibiotics in these environments are facilitating the jumping of resistance genes from chromosomes to plasmids so that they can spread, but the underlying mechanism never been directly tested. That's where our work comes in."It's no secret that the rise in antibiotic resistance in human pathogens has coincided with the rise in the use of antibiotics in large-scale industrial endeavors like farming and manufacturing. While the genes that provide resistance appear to be relatively new to these pathogens, some date back millions of years in bacteria living in certain wild ecosystems. Coupled with experiments that show high levels of antibiotics promote the spread of resistance both within and between bacterial species, it's easy to draw the conclusion that human pathogens have acquired these resistance genes from the environment due to its increasing ambient levels of antibiotics.



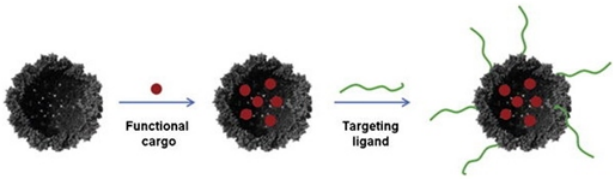
Learning from the single cell: A new technique to unravel gene regulation

How is the activity of genes regulated by the packaging of DNA? To answer this question, a technique to measure both gene expression and DNA packaging at the same time was developed by Franka Rang and Kim de Luca, researchers from the group of Jop Kind (group leader at the Hubrecht Institute and Oncode Investigator). This method, EpiDamID, determines the location of modified proteins around which the DNA is wrapped. It is important to gather information about these modifications, because they influence the accessibility of DNA, thereby affecting the gene activity. EpiDamID is therefore valuable for research into the early development of organisms. The results of the study are published in Molecular Cell on April 1, 2022.



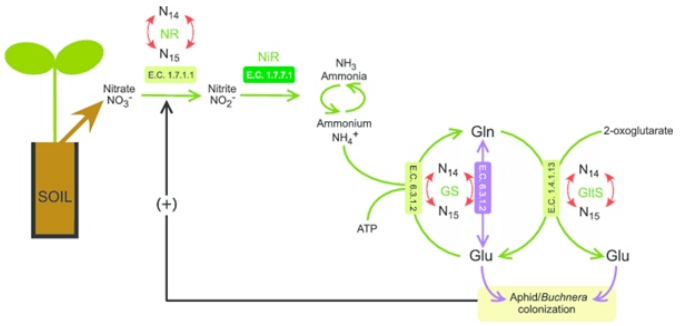
Shape shifting volcano virus points to new ways to deliver drugs, vaccines

From hot volcanic springs where the water is nearly boiling acid, scientists have discovered how lemon-shaped viruses got their form. And that discovery could lead to new and better ways to deliver drugs and vaccines.While the vast majority of viruses are either rod-like or spherical (such as the corona virus responsible for COVID-19), scientists have been puzzled by the unusual forms of viruses found in some of the harshest environments on Earth.The researchers were studying one such virus when they discovered it has strange properties that let it alter its shape. While it normally resembles a lemon or spindle, the virus can grow tails. The structure that lets it do that, the scientists realized, likely explains how ancient rod-like viruses gave rise to all the spindle-shaped viruses seen today."We can now understand a new principle in how proteins can form the shell that packages the DNA in a virus," said lead researcher Edward H. Egelman, PhD, of the University of Virginia School of Medicine. “This has implications for not only understanding how certain viruses evolved but potentially can be used for new ways to deliver everything from drugs to vaccines.”



New protein discovery reveals the mechanisms of nitrogen assimilation in plants

A collaborative research group has discovered the protein that inhibits the formation of organic nitrogen compounds in plants. This protein, if manipulated, could potentially be used to encourage plant growth, improving biomass production and crop harvests. Nitrogen is one of the building blocks of life. Humans need nitrogen to make the amino acids, proteins, and nucleic acids essential for growth, hormones, brain functions, the immune system, and DNA and RNA. Humans, unlike plants, cannot synthesize organic nitrogen molecules. Instead, we rely on plants for our nitrogen intake. Plants utilise nitrate or ammonium in the environment to synthesize organic nitrogen molecules in a process called nitrogen assimilation. Crop production relies on nitrogen fertilizers to improve the efficiency of nitrogen uptake in crops. Still, the regulatory mechanisms behind nitrogen assimilation have continued to elude scientists.



For the first time, India opens doors for genetically modified soyameal imports for poultry

For the first time ever, India will import 15 lakh tonnes of genetically modified (GM) soyameal to help the poultry industry tide over the crisis of high feed prices. The environment ministry has allowed imports as the 'crushed cake does not contain any living organism. The Food Safety and Standards Authority of India (FSSAI) said anything that is non-food is not within its ambit. Importers, including corporates like Godrej Agrovet NSE -1.13 %, said imports will arrive in 10 days as there is severe shortage in the country while global prices are 40% of local prices. India has been importing GM soyabean oil and also consumes locally producd GM cotton seed oil.

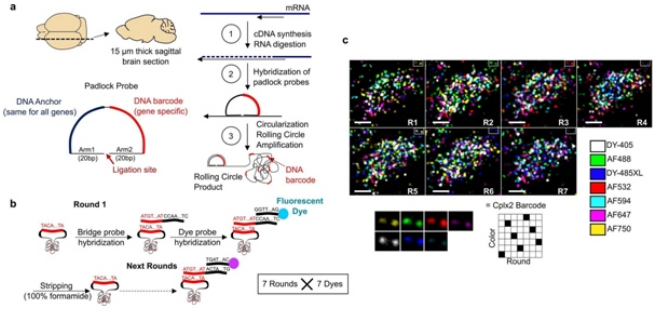
The industry expects import contracts for at least a million tonnes of soyameal will be signed immediately. "The chicken business has been badly hit due to the commodity prices and covid. Now, the demand has started coming back. However, raw material prices increased from Rs 40/kg a year ago to Rs 95/kg. The shortage of soyameal is so big that we expect at least 10 lakh tonnes of soyameal will be immediately imported into the country," said Balram Yadav, managing director, Godrej Agrovet.

India ranks third in Egg, eighth in meat production: Economic Survey 2021-22

India ranks third in production of eggs and eight in meat production in the world, said The Economic Survey 2021-22 . Egg production in the country has increased from 78.48 billion in 2014-15 to 122.11 billion in 2020-21 (Provisional) taking the per capita availability of eggs to 91 eggs per annum in 2020-21 (Provisional).

Meat production in the country has increased from 6.69 million tonnes in 2014-15 to 8.80 million tonnes in 2020-21 (Provisional), the survey said.

New approach to transcriptomics reveals properties of 35 neuron subtypes in mice



A team of researchers from University College London, Columbia University and Oxford University has developed a new approach to conducting transcriptomics and has used it to reveal the properties of 35 neuron subtypes in mice. The group has published their research in the journal *Nature*. Hongkui Zeng and Saskia E. J. de Vries with the Allen Institute

for Brain Sciences have published a News & Views piece in the same journal issue outlining the work done by the team.One of the goals of biological scientists is to learn how the brain works, in other animals and in humans. Considering the complexity of the brain, the goal is ambitious to be sure. But scientists believe that it can be done by carrying out a variety of step-by-step studies that seek to explain the various parts of a given brain to describe what they do. Part of that effort involves creating a catalog of brain cell types and subtypes.One aspect of cell type classification involves deciphering and recording the repertoire of expressed genes—a process called transcriptomics. From this comes the identification of the roles that each of the individual genes play in brain circuitry. To that end, researchers have used various techniques to conduct transcriptomics (which have to be carried out on live tissue) to learn more about regions of the brain, such as the mouse visual cortex. To date 95 groups have been identified.In this new effort, the researchers have developed a new way to carry out such work—they call it coppa FISH, and it is capable of determining the expression of 72 genes at once from a thin slice of brain tissue. It involves combining in vivo two-photon calcium imaging with conventional transcriptomic methods. The resulting expression profiles for the genes can then be used to map the transcriptomes to cell identities.The researchers used the technique to obtain new data for 1,090 interneurons, involving 35 neuron subtypes. In so doing they found that certain transcriptomic principles could be used to predict the activity of other cell types, resulting in a transcriptomic axis that correlates with some types of cell properties. The researchers suggest their technique could be used in other regions of the brain as well.

Nanocellulose may help wild blueberry yield when applied with fertilizer, study finds

Nanocellulose may help increase the yield from wild blueberry plants when used with liquid fertilizer applied to leaves, according to a new University of Maine study. Nanocellulose, a natural polymer derived from trees and plants that has many desirable properties, is used in research and development for a myriad of applications, such as packaging materials, building products, medical supplies, paint, cement, food containers and much more. Previous studies have also found that nanocellulose can improve the adherence of foliar-applied fertilizers and pesticides, which are used directly on leaves; and nanocellulose could make it easier for plant leaves to retain and absorb nutrients.The team found an increase in yield among common lowbush blueberries that received the nanocellulose-infused fertilizer, albeit not significantly. Researchers believe the increased production resulted from the nanocellulose reducing the particle size of nutrients from the fertilizer, making it easier for the blueberry leaves to absorb them and facilitating an uptick in consumption. Further investigations need to be conducted to determine whether increasing the amount of nanocellulose in the fertilizer will result in an even greater yield from common lowbush blueberries, according to researchers. The effects it could have on yield for velvet-leaved lowbush blueberries also should be examined.

NATIONAL AND INTERNATIONAL CONFERENCES

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   World Congress on Medical and Aromatic Plants -Gomel, Belarus
2) **01st Mar,2021**
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3) **01st Mar,2021**
   International Congress on Cell Science and Molecular Biology -Peshawar, Pakistan
4) **01st Mar,2021**
   International Congress on Cell Science and Molecular Biology -Swakopmund, Namibia
5) **01st Mar,2021**
   International Conference on Environment and Life Science -Gomel, Belarus
6) **08th Mar,2021**
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   International Conference on Environment, Agriculture and Biotechnology (ICEABT) -Taipei, Taiwan
9) **08th Mar,2021**
   International Conference on Food Technology, Agriculture and Fisheries (ICFTAF) -Hanoi, Vietnam
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14) **08th Mar, 2021**
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should be in the following format.

Rynold, M.P. (1994). The Archaean grey gnesisses and the genesis of continental crust. In: Archean Crustal Evolution (ed. Candie, K.C.) Elsevier, Amsterdam, pp. 205–259.

Sengar,R.S. (2013). Estimation of population growth and extinction parameters from noisy data. Ecol. Appl., 13 : 806–813.

Sirova, D., Adamec, L. and Verba,J. (2013). Enzymatic activities in trops of four aquatic pieces 3 of the Carnivorous genus Vlricularia, New Phytology 159(3) : 669-675.

Sengar, R.S., Sharma, A.K., Chaudhary, R. and Kureel, R.S. (2009). Biodiesel plant Jatrophaneed for future.

Procedings of ..5th World Congress of Cellular & Molecular Biology (WCCMB, 2012).., November 02- 06, School of Biotechnology, Devi Ahilya University Indore, India & World Society of Cellular & Molecular Biology, France. 142-143.

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