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Effect of Sulphur Oxidizing Bacteria (SOB) on sulphur nutrition in Sesame (*Sesamum indicum*)

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ABSTRACT

Sulphur (S) plays an important role particularly in the nutrition of Oilcrops as it is a key element of S containing amino acids. Sulphur is a constituent of three amino acids commonly found in plants viz., cystine, cysteine, and methionine, which are essential components of proteins. Sulphur increase the oil content and gives pungency to oil as it forms certain disulphide linkages. Oil seeds crops like sesame, sunflower, groundnut etc., require more sulphur than cereals as their oil storage organs are mostly protein, rich in sulphur. Certain types of bacteria are able to oxidize, reduced form of sulphur which is unavailable to plants into available form. These types of bacteria are called as Sulphur Oxidizing Bacteria (SOB), which can live in very different environments, from deep in the ocean to freshwater marshes. One of the best examples of SOB is a genus *Thiobacillus* i.e., *Thiobacillus thiooxidans, Thiobacillus ferrooxidans, Thiobacillus thioparus, Thiobacillus novellus*. SOB generally improves the production or the conversion of the elemental sulphur to the sulphate ($SO4^{-2}$) for absorption and results in the plant growth promotion and production process. Because of applying Sulphur Oxidizing Bacteria (SOB) to oilcrops, an amount of available sulphur to the plant is increased, this in turn helps to enhance the oil seed production and productivity.

Key words: Sulphur nutrition, sesame, sulphur oxidizing bacteria (SOB), oil seed production and productivity.

Sesame (Gingelly) is called as queen of oil seeds. The oil seeds are important constituent in human dietary system next to carbohydrate and protein (Pal and Gangwar, 2004). Among the oil seed crops, sesame has the highest oil content of 46-64% with 25% protein. Sulphur requirement is equal to that of phosphorus (Scherer, 2001), its essential for the growth and development of a crop, it plays a key role in plant metabolism, indispensable for the synthesis of essential oil, and also in chlorophyll formation (Ajai singh *et al*., 2000) and required for development of cells and also increases the cold resistance and drought hardiness of crops especially for oilseed crops. Sesame seed, commonly known as til in India is largely produced for its oil and also used as a flavouring agent. The seeds come in several colours like red, white, black, yellow, depending upon the variety of the seeds. The sulphur should increase the oil content around 55%. They are also a good source of magnesium, calcium, iron, phosphorus, vitamin B1, selenium, and zinc. In addition, sesame seeds are a good source of both dietary fibre and mono unsaturated fats. At present national average yields of sesame is 303 kg ha-1, which needs to be increased at least 1.2 and 1.5 tonnes by 2025 as reported by Hegde (2008). Use of sulphur free fertilizers, heavy sulphur removal by the crops under intensive cultivation and neglect of sulphur replenishment contributed to widespread of sulphur deficiencies in arable soils. Sulphur has become one of the major limiting nutrients for oilseeds in recent years due to its widespread deficiency (Hegde and Murthy, 2005). Sulphur-deficiency symptoms are more frequently observed in crops at early stages of growth, because it can be easily leached from the surface soil. In acute deficiency of sulphur, flowering and seed formation is greatly reduced, resulting in poor yield and oil content. To overcome this deficiency, sulphur fertilizers can be applied which can increase the pungency of the oil content. Sulphur also plays an important role in the chemical composition of the seeds. Sulphur is the component of the amino acids, cystine, cysteine and methionine, needed for chlorophyll and also increases the oil percentage.

Sulphur

Sulphur is the major nutrient for the plant growth and it is available in the larger quantities in the unavailable form. The prime sulphur source of soil is considered to be sulphides of metals in plutonic rocks. As these rocks are exposed to weathering process, the sulphide minerals decompose and also oxidized to sulphates (Whitehead, 1964). These sulphates are then precipitated as soluble and insoluble salts in arid and semi-arid climates, absorbed or reduced by living organism to sulphide or elemental sulphur under anerobic condition (Freney, 1986). Plant sulphur nutrition depend primarily on the uptake of inorganic sulphate. However, the recent research has demonstrated that from the sulphate ester and sulphonate pools of soil sulphur are also plant-bioavailable, probably due to inter conversion of carbon–bonded sulphur and sulphate ester–sulphur to inorganic sulphate by soil microbes (Kertesz and Mirleau, 2004).

State of sulphur in soil

In soil, sulphur mainly occurs in the form of elemental sulphur, mineral sulphides, sulphates, hydrogen sulfide and organic sulphur compounds (Blair *et al.*, 1991); Stevenson and Cole, 1999; Takkar, 1988). Since most of the sulphur in soils of humid regions is present in organic compounds, their transformation is important in the conversion of sulphur to substances available to plants. Much of the organic sulphur may be released as incompletely oxidized inorganic substances such as sulfide, elemental sulphur, thiosulphate, tetrathionate and Pentathionate, and it is presumed that these are generally oxidized to sulphate before being absorbed by plants.

Transformation of sulphur in soil

The major form of the sulphur taken by the plant and microbe was sulphate. Sulphur usually produced by the weathering of the originally bound sulphur in the rocks and soil system. The conversion process of elemental sulphur is very important for the formation of the available form of sulphur to the plants (Singh, 1988). Tandon (1989) proposed that sulphur will be abundant only in the fine and coarse textured soils. Sulphur conversions in soil occur mainly by the activity of the soil a microorganism which involves the processes of oxidation, immobilization and biotransformations. Sulphur in the soil is always available in its reduced form as sulphide. The oxidation of the metal sulphides mainly involves *Thiobacillus ferrooxidans* in the oceans. (Kapoor and Mishra, 1989), experiential that sulphur was very quickly oxidized in the field at the pH 8.0 and the oxidation process was increased by inoculation with *T. thiooxidans*..

Organic sulphur transformation in soil

Majority of S (>95%) in soil is bound to organic molecules that can be indirectly available to plants (Kertesz and Mirleau, 2004). Organic sulphur not directly bound to carbon(c), which is reduced to H2S by hydriodic acid (HI) and organic sulphur directly bound to carbon is composed primarily of sulphate esters (C-O-S)., such as phenol sulphate, sulphate lipids and ulphated polysaccharide among others. The fraction of organic S directly bound to C consists of the S-containing amino acids, thiols, disulfides, sulfones, and sulfonic acids. Elemental sulphur (S0) is the immediate product of hydrogen sulfide (H2S) oxidation and the most stable form of S (Suzuki, 1999). Soil that has high organic matter appear to have proportionally less ulphur present in non-carbon bonded forms. Carbon-bonded organic sulphur fraction of soils also includes oxidised forms such as sulphinates, sulphonates, sulphoxides and sulphones. Organic sulphur, an important reservoir from the available sulphur can be released to sustain plant growth.

Inorganic sulphur transformation in soil

Inorganic sulphur is the easily available fraction for root uptake, but it accounts for the average 5% of the total S in the soil. And these inorganic forms of sulphur are generally less abundant than the organic sulphur (Halstead and Rennie, 1977). The most predominant form of inorganic sulphur in well dried soil is the sulphate accounting for <1% of total sulphur (Williams, 1972). Inorganic sulphur was first categorized by (Halstead and Rennie, 1977). The classification mainly includes the easily soluble sulphate, adsorbed sulphate, insoluble sulphate; insoluble sulphate co-precipitated with CaCO3 and reduced inorganic compounds. Dry areas may easily uptake and accumulate easily soluble sulphate that accounts for 1 % to 10 % of total soil sulphur. Soluble sulphate content varies depending on the depth of the soil, which being low in sandy sub soils and very high in calcareous. Adsorbed sulphate is the major form present in the humic soils which contain substantial amounts of hydrated aluminium or ferric oxides. Soils showing high level of adsorbed sulphates are red yellow podzols and latosols. Adsorption mechanisms are complex and generally not completely understood; perhaps it involves anion exchange processes, complex formation and surface effects on clay compounds. In calcareous soils, sulphate might not be available to plants because of co- precipitation with CaCO3 (Halstead and Rennie, 1977). Under anaerobic conditions i.e. waterlogged conditions or due to the availability of excess organic matter, hydrogen sulphide will be produced the bacterial sulphate reduction process or by decomposition of organic matter. Sulphide accumulation is one of the major factors in strip- mine soils, tidal mud flats and also in marine, estuarine and freshwater.

Mechanism of oxidation of sulphur:

The transformations of sulphur in the process of oxygen undergoes metabolism of the organism, the end-product is sulphuric acid; an increase in oxygen tension increases the mechanism of oxidation of sulphur by the organisms. It is also possible that oxygen from air is not the only source; as pointed out above this particular organism derives its energy not from carbohydrates but from the oxidation of sulphur and is autotrophic in nature. Like green plants, the autotrophic organisms use the carbon dioxide from air for structural purposes, but unlike plants, these organisms accomplish it without the intervention of the photochemical reactions. The process of assimilation of carbon dioxide is accompanied by the splitting of oxygen, which may also be used by the sulphur organisms in the process of oxidation (Friedrich *et al.,* 2001).

Sulphur Oxidizing Bacteria (SOB)

Sulphur plays a dominant role in the assembly of proteins, vitamins and enzymes and it is one of the essential elements of the essential amino acids cystine, cysteine and methionine. It is mainly available in the form of elemental sulphur, mineral sulphides, hydrogen sulphide and organic sulphur. Since most of the 'S' in soils is present in organic compounds, sulphur cannot be absorbed as such by the plants. Plants take up sulphur in the form of sulphate. Sulphur oxidizing bacteria efficiently converts form of elemental sulphur, organic sulphur (unavailable form to the plants) into sulphate form (available form to the plants) by sulphur oxidation process. Inorganic acid produced by the bacterium helps in pH reduction of problem soil. Several commercial formulations of elemental sulphur are available which has a variety of uses as a soil amendment. The oxidation of sulphur to H2SO4 is particularly beneficial in alkaline soils to reduce pH, supply SO4 to plants, make P and micronutrients more available and reclaim soils (Burns, 1967). However, sulphur oxidation is often npredictable in many soils, incuding some from New Mexico (Vitolins and Swaby, 1969). Sulphur oxidization process mainly improves the soil fertility and thereby results in the formation of sulphate, which can be utilized by the plants, while the acidity produced in sulphur oxidation helps to solubilize plant nutrients and lowers the pH of alkaline soils (Wainwright, 1984). Sulphur oxidizing bacteria (SOB) generally improves the production or the conversion of the elemental sulphur to the sulphate ($SO4^{-2}$) for absorption and results in the plant growth promotion and production process (Smith and Hawkesford, 2000). SOB play an important role in oxidation of toxic sulphides in mangrove ecosystem and act as a key driving force for all sulphur transformations (Behera *et al.,* 2014). Thirty sulphur oxidizing bacteria were isolated from six different location of mangrove soil. From the qualitative screening it was found that out of the thirty bacterial isolates, twelve isolates could efficiently reduce the pH of the medium up to 4.2 from the initial pH 8.0. Their sulphate ion production abilities were in the range of 125 mg ml^{-1} to 245 mg ml^{-1}. Their sulphur oxidase activities were in the range of 11.6 to 126.83 U ml^{-1} min^{-1}. Biological oxidation of hydrogen sulphide to sulphate is one of the major mechanisms of sulphur cycle on earth. Other than hydrogen sulphide, bacteria have the ability to oxidize sulphur, sulphite, thiosulphate and various polythionate under alkaline neutral or acidic conditions. Aerobic sulphur oxidizing bacteria belongs to genera like *Acidithiobacillus* and *Thermithiobacillus* (Kelly and Wood, 2000). Sulphur may be oxidized by many groups of bacteria such as *Thiobacillus,* the most promising and characteristic group of bacteria performing the oxidative part of sulphur transformation in soil (Waksman and Joffe, 1922). Inoculation of *Thiobacillus* on Starkey broth increases the sulphur oxidation rate. Sulphur oxidizing bacteria are used as biofertilizers for reclamation of sodic soil. In rock biofertilizers from P and K rocks with the sulphur inoculated with *Acidithiobacillus* have been applied to different regions of semiarid and arid regions get the results also good.

Organisms involved in S Oxidation

Thiobacillus plays a significant role in sulphur oxidation in the soil (Vidyalakshmi *et al.,* 2009). The oxidation process is the chief process of S-cycle and marks in the development of sulphate which can be used by plant and it will progress the fertility of alkali soils. The soil microbial biomass is the input for all sulphur transformations. Aerobic and obligate chemoautotrophic *Thiobacilli* have been considered as the most important group and have been intensively studied by many scientists. Pepper (1975) compared the relative importance of two strains of *Micrococcus* (heterotrophs) to that of the autotroph *Thiobacillus thiooxidans*. He concluded that "heterotrophic oxidation of reduced inorganic sulphur compounds can be effective as autotrophic oxidation". Obligate chemoautotrophs use S compounds as electron donors and carbon dioxide as their sole carbon source. *Thiobacillus thiooxidans*, an obligate autotroph, was first isolated by (Waksman and Joffe, 1922) from a compost of pulverized rock phosphate, sulphur, and soil. This organism is also unique in that it is tolerant to extremely acid conditions (Starkey, 1966). It develops best under acid conditions, p H < 4, and shows little or no development under neutral pH. It is capable of rapidly oxidizing S°. Sulphides, thiosulphates, tetrathionates and sulphites could also be oxidized by this species (Starkey, 1966). *Thiobacillus ferrooxidans* and another obligate chemoautotroph, are also acid tolerant and is capable of oxidizing S°. A distinctive characteristic of this bacterium is its ability to utilize the energy released from aerobic oxidation of ferrous iron under acid conditions (Starkey, 1966). *Thiobacillus thioparus* was widely distributed in soils, grows at nearly neutral reactions, and oxidizes sulphur to sulphide. *Thiobacillus novellus* is an exception because of its ability to use organic compounds (Vavra and Frederick, 1952), hence it is referred to as a facultative autotroph.

Sox *gene* in sulphur oxidizing bacteria

The gene cluster that codes for the capacity of the SOB isolates to oxidize the elemental sulphur (Sox) comprised of about two transcriptional units with 15 genes in *Paracoccus pantotrophus*. Seven genes, SoxXYZABCD, which always codes for the *in vitro* production of the essential proteins. These genes and SoxFGH are induced by thiosulphate. Four open reading frames (ORFs), ORF1, ORF2, and SoxVW, which was mainly located in the upstream position of the Sox gene cluster. As would be expected from previous biochemical work with *T. denitrificans*, its genome contains a diverse complement of genes encoding enzymes that catalyze inorganic sulphur compound oxidation and energy observation and energy conservation by both (substrate level and electron transport linked phosphorylation). In *Thiobacillus denitrificans*, genes showing various levels of sequence identity to sox genes of this α-proteobacteria have been detected, but gene clusters of the length found in facultatively chemolithotrophic, aerobic, thiosulphate – oxidizing bacteria donot occur (Beller *et al.,* 2006).

Effect of sulphur levels on sesame crop:

The experiment was conducted and the suitable crop of sesame was sown during rabi season at sodic soils with four levels of sulphur i.e. 0, 20, 40 and 60 kg ha-1 and the sulphur was applied with different doses that can increases the plant height, (Sharma and Gupta, 2003). The different doses for increasing the plant height. Duary and Mandal (2006) conducted an experiment on response of summer sesame to varying levels of sulphur i.e. 20, 40, 60 kg S ha-1 and that plant height (129.10 cm) was increased significantly with application of 40 kg S ha-1 and it was significantly superior over control, 20 and 60 kg S ha-1 on sodic soil. An experiment was study about the effect of varying levels of sulphur on growth and yield attributes of Sesame and revealed that application of sulphur @ 40 kg ha-1 have significantly increased plant height (74.83 cm) and it was on par with 20 and 50 kg S ha-1during the rabi season on sodic soil (Jadav *et al*., 2010). The plant height was increased significantly with application of 40 kg S ha-1 and it was on par with application of 20 kg S ha-1 on medium calcareous soils during rabi season (Vaghani *et al*., 2010). The sulphur level increases with the plant height. Application of 50 kg S ha-1 recorded the highest plant height (130 cm) over control, 10, 20, 30 and 40 kg S ha-1 in sesame during summer season on sodic soil (Tahir *et al.,* 2014).

Dry matter production:

The effects of sulphur on nutrient uptake yield and food value of sesame (*Sesamum indicum*) increases the biomass production, of sesame crop with sulphur levels up to 60 kg / ha (Kundu *et al*., 2008). Dayanand and Shivram (2002) proved that, application of sulphur @ 60 kg / ha. has the effect of sulphur on growth and yield attributes of sesame increased the dry matter production, (Sharma and Gupta, 2003). The observation on the effects of sulphur at 0, 20, 40, 60 and 80 kg ha^{-1} applied alone or in combination with farmyard manure, phosphate, potash and urea was applied, the seed yield as well as S uptake of sesame (Maragatham *et al*., 2006). The highest dry matter was observed for applied @ 40 kg ha^{-1}. Significant increase in plant dry matter (11.3g) was observed with increasing sulphur level from 0 to 20 kg S ha^{-1} in sesame (Mondal *et al*., 2012).

Number of capsules per plant:

The effect of nitrogen and sulphur applied @ 40 kg ha-1increased the number of capsules per plant over the control (Nagavani *et al*., 2001). Duary and Mandal (2006) observed that number of capsules plant-1 (55.00) was increased significantly with application of 40 kg S ha-1 over control, 20 and 60 kg S ha-1 of sesame during summer season on sodic soils. The combined application of 40 kg S ha-1 along with recommended dose of fertilizer (60: 30: 30 kg N, P, K ha^{-1}) increased the number of capsules per plant (82.00) over control, 20, 60 kg S ha^{-1} (Kundu *et al.,* 2008)..

CONCLUSION

Inoculations of sulphur oxidizing bacteria with sesame seeds oxidized the reduced sulphur compounds and make them available to plants in sulphate form, which further result in improvement in plant growth parameters such as length, weight, no. of capsules per plant, seed weight, oil content, dry matter production and chlorophyll..

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