



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2019; 8(11): 49-52

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www.thepharmajournal.com

Received: 23-09-2019

Accepted: 27-10-2019

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Role of sulphur oxidizing bacteria inoculated mustard in livestock production via effect on mustard growth

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Abstract

Sulphur is a vital element for plants next to nitrogen (N), phosphorus (P), and potassium (K). It is an important constituent of proteins, enzymes, vitamins, lipids, carbohydrates, and other biomolecules. Sulphur is required for growth and development of plants especially in the crop production. Sulphur undergoes a number of biological alterations in nature carried out exclusively by microbes through sulphur cycle. Oxidation of sulphur is the most important step of S cycle which improves soil fertility. It results in the production of sulphate, which can be easily used by the plants, while the acidity produced by oxidation step helps to solubilize plant nutrients and thus improves soil health. Sulphur deficiencies in soils of tropical and subtropical regions have been recognized for many years and have been reported from over 70 countries, including India.

Use of sulphur oxidising bacteria has been reported to enhance mustard growth, which is an important protein supplement in animal feed. SOB's promote mustard growth via increase in the height, weight, no. of siliquae, 100 seed weight, oil content, leaves protein content, leaves chlorophyll content, viable rhizospheric bacterial count. Hence, the mustard inoculated with SOB has a higher quality. Use of such SOB inoculated mustard as a protein supplement in animal feed can have a favourable outcome in terms of animal growth and production.

Keywords: Mustard, animal feed, sulphur oxidizing bacteria

Introduction

Sulphur (S) is recognized as the 4th key nutrient in addition to nitrogen (N), phosphorus (P) and potassium (K), which limits the growth of plants. Sulphur has a role in the synthesis of proteins, oils, vitamins and flavoured compounds in plants. The amino acids methionine and cysteine and cystine contain 21, 26 and 27% S respectively, which are involved in the formation of proteins. Almost 90% of S is present in these amino acids only (Tandon and Messick, 2002) [32]. Sulphur plays important part in the formation of glucosides, chlorophyll, glucosinolates (mustard oils), initiation of enzymes and sulphhydryl (SH⁻) that are sources of pungent smell in oils and onion (Mengel and Kirkby, 1987) [25]. It is also required for synthesis of number of essential vitamins such as biotin and thiamine (B1) and co-factors like Co-enzyme A, Sadenosyl-L-methionine, molybdenum cofactor (MoCo) and lipoic acid (Kertesz and Mirleau, 2004; Jamal *et al.*, 2010) [19, 16].

Though S is one of the vital nutrients required for plant growth with having similar requisite as phosphorus, this nutrient received negligible attention for so many years. The areas of sulphur shortage are becoming prevalent all over the world due to intensive agriculture, very less sulphur returns with farmyard manure (FYM), high yielding varieties and use of chemical fertilizers having very low amount of sulphur (Jamal *et al.*, 2010) [16]. The deficiency of sulphur is emerging fast in areas where sulphur free fertilizers like diammonium phosphate (DAP) and urea are being used continuously.

Sulphur undergoes so many biological conversions in the nature, which are carried out absolutely by microorganisms. Most of the S is absorbed by plant roots in the form of sulphate (SO₄⁻²), which further undergoes a series of conversions before its incorporation into the original S requiring compounds (Katyal *et al.*, 1997) [17]. The transformations of inorganic sulphur compounds in nature have been formalized in the so-called sulphur cycle. The physiologically different types of microorganisms, which comprise of both heterotrophic and autotrophic mode of nutrition, participate in S cycle. The bacteria, which are having capacity to oxidize the reduced forms of inorganic S compounds with SO₄⁻² as a final product, are generally known as sulphur oxidizing bacteria (SOB). Besides their contribution in agricultural practices, the sulphur oxidizing microorganisms also play an important role in removal of poisonous hydrogen

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sulphide (H₂S) from the atmosphere. The most popular, SOB generally belongs to some genus like *Thiothrix*, *Thiobacillus*, *Beggiatoa*, *Thiomicrospira*, *Desulphuromonas* and *Achromatium* (Das *et al.*, 1996) [9]. However, the oxidation process of S containing compounds is not limited to the true sulphur bacteria only; it takes place in the heterotrophic bacteria as well. Most of these heterotrophic bacteria, which are isolated from different kind of environments, belongs to the genera *Escherichia coli*, *Pseudomonas*, *Alcaligenes* and *Xanthobacter* (Starkey, 1935; Kuenen and Beudeker, 1982) [31, 20].

Sulphur is an important constituent of mustard oil, leaves and its deficiency caused a significant reduction in yield and oil content of mustard (Aulakh, 2003) [4]. In Western European countries, occurrence of sulphur deficiency has been reported in members of family Brassicaceae (Scherer, 2009) [30]. Rapeseed and mustard are the third important oilseed crops after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.) oil in the world. India is holding a leading place in rapeseed economy of the world with second and third rank in area and production, respectively. Mustard is among one of the peculiar edible oil seed crop comprising an area, production and mustard productivity of 5.76 million hectares, 6.82 million tonnes and 1184 kg/ha respectively during the year 2015-16 (Anonymous, 2016) [3]. It belongs to the family *Brassicaceae* (*Cruciferae*) and order *Brassicales* and the productivity of Haryana, Gujarat, Rajasthan, UP and MP was above 1000 kg/ha (Rathore *et al.*, 2018) [29].

Use of such Sulphur enriched mustard in animal feed can have various positive growth and production implications.

Sulphur pools in soil

The main forms of S present in nature are sulphide and sulphate in water or soil and sulphur dioxide in the aerial environment; whilst sulfoxides, elemental sulphur, thiosulphate and polythionate plays a lesser but significant role. Sulphur can be divided mainly into two forms- (i) organic (ii) inorganic sulphur compounds. Most of the sulphur in soils is found in the form bounded to the organic molecules (~90%), which is divided in two main groups: in the first group S atom is present in the oxidized state and in the other group, it is present in the reduced form (Chaudhary, 2018) [6]. In spite of the fact, that plants uptake sulphur mainly in the form of sulfate, organic S pools are also significant sources of sulphur for plants during their growing season (De Bona and Monteiro, 2010) [10].

The inorganic sulphur usually accounts for less than 10% of total sulphur (Eriksen, 2009) [12] generally found in the form of elemental sulphur, sulfite, tetrathionate, hydrogen sulphide and thiosulphate. The major natural sources of reduced sulphur compounds include hot springs, Volcanoes and hydrothermal vents (Madigan *et al.*, 2009) [24]. Apart from this, industrial activities like petroleum, natural gas extraction, production of rayon textile, paper and chemical manufacturing, agricultural and waste disposal are also responsible for adding inorganic sulphur compounds to the atmosphere (Park *et al.*, 2002) [28].

Sulphur oxidizing microorganisms

Microorganisms having capability of oxidizing reduced inorganic sulphur compounds with sulphate as an end product are known as sulphur oxidizing microorganisms (SOM) Chaudhary *et al.*, 2018. Microorganisms play a significant role in S transformations. Sulphate is taken up as a nutrient and reduced to sulphide, which is then incorporated into sulphur-containing amino acids and enzymes (Friedrich *et al.*, 2001)

[13]. Production of SO₄²⁻ from either SO or thiosulphate in the soils is thought to be principally a microbiological-mediated process takes place in aerobic conditions by many different microorganisms and fungi (Barbosa-Jefferson *et al.*, 1998) [5]. The bacteria, those are having capacity of oxidizing the reduced forms of inorganic S compounds into sulphate as the finished product, are named as SOB (Das *et al.*, 1996) [9]. These can be isolated from broad range of environments and are dispersed over α , β and γ -proteobacteria (Dam *et al.*, 2007) [8]. The SOB can oxidize polythionate, thiosulphate, H₂S, sulfite and so by using oxygen as final e- acceptor (Graff and Stubner, 2003) [15].

Effect of inoculation of SOB and other microorganisms for plant growth

The microorganisms present in soil improve the plant growth by providing nutrients and defending them against stress and pathogens.

Gaind and Gaur (1991) [14] tested many phosphate solubilizing microorganisms (PSM) for their effect on growth of mungbean. When these strains were inoculated with seeds of mung bean, they showed a better establishment of temperature tolerant strains as, revealed by counting the rhizosphere population. Kumar *et al.* (2001) [21] conducted a pot experiment in the green house to examine the establishment of *Azotobacter chroococcum*, in the rhizospheric parameter. Their effect was studied on growth parameters of three genetically divergent wheat cultivars (*Triticum aestivum* L.). The inoculation of wheat seeds with *A. chroococcum* showed better response in comparison to that of control and mutant strains of *A. chroococcum* showed much higher increase in grain (12.6%) and straw (11.4%) yield over the control. The survival mutant strain was more (12–14%) in the rhizosphere and performed better terms of grain yield (14.0%) and root biomass (11.4%) over the control.

Anandham *et al.* (2007) [1] demonstrated the use of *Rhizobium* co-inoculation with the sulfur (S)-oxidizing bacterial strains. Clay-based pellets of *Thiobacillus* were formulated (2.5107 cfu/g pellet) and their efficacy to enhance plant growth was tested in groundnut under pot house and field conditions with sulphur-deficit soil. Experimentation in pot house yielded promising results on groundnut by increasing the plant biomass, nodule number and pod yield and co-inoculation of *Thiobacillus* sp. (60 kg/ha) with *Rhizobium* under field condition also recorded significantly improvement in all characteristics. Also inoculation of SOB increased the available S of soil from 7.4 to 8.43 kg/ha and oil content of groundnut.

Anandham *et al.* (2008) [2] tested the thiosulfate oxidizing bacteria for their traits related to plant growth promotion. In gnotobiotic experiments, *Pandoraea sputorum* ATSB28, enhanced the primary root length of canola by 166% and inoculation of *Pandoraea* sp. strain ATSB30 with RP and thiosulfate significantly increased the water extractable-P (1147 $\mu\text{g P g/ RP}$) and bicarbonate extractable-P (1144 $\mu\text{g P g/ RP}$) on 45th day.

Wani and Khan (2010) [34] studied the effect of plant growth promoting rhizobacteria on chromium presence in soil and chickpea growth. The inoculation of chromium reducing and plant growth promoting *Bacillus* species PSB10, significantly improved the growth, nodulation, leghemoglobin, chlorophyll, seed yield and protein of grains in comparison to that of plants grown in the absence of any bio-inoculant. The strain also found to significantly decrease the uptake of chromium in

roots, shoots and grains of crop compared to plants grown without any bio-inoculant.

Mustard an important oil crop

Brassica juncea, commonly known as Indian mustard, brown mustard, leaf mustard, Chinese mustard, Oriental mustard and vegetable mustard, is a species of mustard plant. Its scientific classification is; Kingdom- Plantae; Phylum-Angiosperms; Class-Eudicots; Order- Brassicales; Family- Brassicaceae; Genus-*Brassica*; Species-*B. Juncea*. *Brassica juncea* species further can be divided into four sub-species- *Integrifolia*, *Juncea*, *Napiformis* and *Taisai* (Kaushik, 2015) [18]. Although there are almost no archeological records for this crop but it was well established crop in Roman and Hellenistic times. According to Encyclopædia Britannica, mustard was grown by the Indus Civilization of 2500-1700 BCE (Encyclopædia, 2016) [11]. Mustard is of great importance as it is used in many ways. The leaves of mustard are eaten as a vegetable in Africa and many parts of Asia; they are mostly torn, cooked and served as a complementary dish with the main food (Kaushik, 2015) [18].

The essential oil of mustard acts as a very strong stimulating agent for the digestion circulation and excretion systems. Mustard oil is recommended for people having less hunger, as an appetizer for boosting the hunger. The enhancement in hunger results due to irritation of the inner lining of intestine and stomach, thus stimulating digestive juices and a person feels hungry. Mustard oil is also a good antibacterial agent helps in fighting bacteria internally and externally. The mustard oil also acts as antifungal agent because of its allyl isothiocyanate component, which does not allow fungus to grow. The mustard oil also acts as hair vitalizer, insect repellent, cordial, antirheumatic, diaphoretic and a tonic substance (Mithen 2001; Vig *et al.* 2009; Milani *et al.*, 2014) [27, 33, 26].

Biological effects of dietary intake of mustard in animals

The composition of mustard cake (MOC) varies with the variety, growing conditions and processing methods. These cakes contain 1–12% ether extract depending upon the method of oil extraction. The protein has a good balance of essential amino acids and relatively high methionine content. However, low palatability of mustard cake is said to be the main problem for its utilization in ruminant diets. This problem is attributed to its glucosinolate content which yields hot and pungent metabolites upon hydrolysis due to the action of endogenous enzymes. Ruminants appear to be less susceptible to the toxic effects of glucosinolates compared with pigs and poultry. This is probably the result of glucosinolates being relatively unhydrolysed in the rumen (Lanzani *et al.*, 1974) [23].

Effect of SOB inoculated mustard in animal production and growth

SOB inoculated mustard can be used in the feed of animals especially as a source of protein for body growth in the form of oilcakes or in feed blocks (Tripathi and Mishra *et al.*, 2006) [36]. Sulphur is essential for wool production and feeding mustard cakes to lambs has been reported to give better performance results (Kumar *et al.*, 2002) [28] and proves to be more economical than the other protein sources.

Conclusion

Inoculation of SOB along with mustard seeds will oxidize the reduced sulphur compounds, thus make it available to plants in

sulphate form and this will result in improvement in plant growth parameters such as height, weight, no. of siliques, seed weight, oil content, leaves protein content and viable rhizospheric bacterial count. And a higher sulphate content in the mustard plant used as a protein source for animals will help boosting their overall performance.

It may be inferred that other protein sources may be replaced with mustard cake without affecting feed intake, nitrogen balance, mineral balance and growth performance of livestock species. The SOB isolates should be further explored in field conditions for mustard and other oil seed crops and their subsequent result on animals via feeding trials.

Acknowledgement

Financial assistance throughout the degree from DST (INSPIRE) Department of Science and Technology, Technology Bhavan, New Mehrauli Road, New Delhi – 110016, is greatly acknowledged.

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