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Potential Role of Organic Matters and Phosphate Solubilizing Bacteria (PSB) on the Growth and Productivity of Fenugreek

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ABSTRACT

A field experiment was conducted during 2009-2011 at the Aligarh Muslim University Agricultural Research Farm, India, to evaluate the efficacious nature of some oil-seed cakes such as neem cake and castor cake, a botanical *Calotropis procera* and phosphate solubilizing bacteria (PSB) *Pseudomonas fluorescens* singly and in various combinations, on the growth and productivity of *Trigonella* plant. Growth parameters included fresh and dry weight, pollen fertility (%), pods plant^{-1}, root-nodule index, nitrate reductase activity, and chlorophyll content. Productivity was calculated in terms of N, P, and K in plant as well as in soil. Although all the parameters were significantly increased in these treatments, single application was comparatively less effective than the combined applications. Among oil-seed cakes, neem cake was found better in promoting plant growth than castor cake, followed by *C. procera* and PSB. Root-nodulation also showed a considerable increase in combined treatments. Maximum growth and productivity were observed in the combined inoculation of neem cake, castor cake, *C. procera* and PSB, as compared to other treatments including inorganic fertilizers and untreated one.

Keywords: Botanical, Oil-seed cakes, *Pseudomonas fluorescens*, *Trigonella*.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.), locally known as Methi, is a multipurpose crop grown in Northern Indian states like Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Haryana, Punjab, Uttar Pradesh and Andhra Pradesh, during winter season. Every part of this plant is utilized as leafy vegetables, fodder and condiments (Khiriya and Singh, 2003). Its seeds are a good source of protein, vitamins, alkaloid *trigonellin*, and essential oil and has an immense medicinal value particularly against digestive disorders (Bhunia *et al*., 2006). Saxena and Ahmad (1983) also reported that importance of Methi as medicinal plant is fast increasing and in recent past, its high market prices have attracted the farmers to grow the crop in various agricultural systems. This crop is cultivated in 35,737 ha with a productivity of 1,000 kg ha^{-1}. However, there is a wide gap between realized yield and potential yield that needs to be bridged by patenting research gap at farming level. The productivity of *Trigonella foenum-graecum* is low because of several constraints. Amongst the production constraints, imbalanced and inadequate nutrition, are considered to be the important limiting factors. Identification of organic and inorganic nutrition is considered to be the first and foremost step for development of production technology. Jat *et al*. (2006) also suggested that productivity of a crop is controlled by many factors, of which mineral nutrition is by and large the most important one, but the application of all the needed nutrients through chemical fertilizers had deleterious effect on soil fertility. Poor

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recycling of organic sources also leads to emergence of multiple nutrient deficiencies (Kumar, 2008).

The indiscriminate and excessive use of chemical pesticides and inorganic fertilizers have resulted in the development of pesticide-resistant insects besides various ecological problems. The remedy to overcome all those problems is the development of integrated nutrient management, in which chemicals and organic sources of plant nutrients are used in combination to sustain crop and soil health. Many organic sources are available but their nutrient supplying capacity is not similar. Sivaprakashan (1991) reported that plant debris, farmyard manure, and compost improved crop productivity by improving nutrient status and soil tilth, besides increasing microbial activity in the soil. For sustaining the desired crop productivity under integrated nutrition system, the nutrient supply through oil-seed cakes, botanicals, and other organic manures not only reduce the dependence on chemical fertilizers but also improve the soil structure, encourage the growth and activity of beneficial organisms in the soil, alleviate the deficiency of secondary and micronutrients, and sustain higher productivity due to improved soil health (Singh *et al*., 2006; Tiwari, 2002).

Biofertilizers also play an important role in integrated system. Further, they are low cost and eco-friendly with tremendous potential for supplying nutrients and can replace the use of synthetic nitrogenous fertilizers by 25-50%, through biological nitrogen fixation (Pattanayak *et al*., 2007). Phosphate-solubilizing bacteria (PSB) species like *Pseudomonas striata* and *Bacillus polymyxa* are also reported to be beneficial in increasing the phosphorus availability in soil and thereby seed yield of pulses (Gupta, 2006). They have also been reported to produce siderophore, antibiotics and exogenous compounds, which directly or indirectly increase the growth of the crop (Registeri *et al*., 2012). Phosphorus is known to enhance symbiotic nitrogen fixation in plant metabolism (Bhatnagar *et al*., 1979). Hence, there is a need to inoculate the crops with effective strains of these microorganisms which improve nutritional status of the soil by application of various oil-seed cakes like castor and neem, a botanical *Calotropis procera* needed for biological nitrogen fixation. However, no such information are available on these aspects. Keeping in view the importance of fenugreek and its lower yield, which seems to be due to nutritional deficiencies, it is, therefore, considered worthwhile to investigate the nutrient source of oil-seed cakes and use of botanicals and phosphate-solubilizing bacteria (PSB) on Methi in field trials.

MATERIALS AND METHODS

Preparation of Field

The experiment was conducted for two consecutive years i.e. 2009-2010 and 2010-2011. The experimental field at Aligarh Muslim University Agricultural Research Farm, India, was thoroughly ploughed and small beds of 6m^2 were prepared leaving 0.5m buffer zones between them. These beds were separately treated with oil-seed cakes of neem (*Azadirachta indica*), castor (*Ricinus communis*); plant parts of a botanical, *Calotropis procera* at 110 kg N ha^{-1}, and with phosphate-solubilizing bacteria (PSB), *Pseudomonas fluorescens,* singly and in various combinations before sowing the seeds. The beds were watered immediately to assist the decomposition of oil-seed cakes and plant parts of botanical, and after 10 days, bacteria–inoculated seeds of Fenugreek (*Trigonella foenum-graecum* L.) cv. UM-33 were sown. For inoculation with PSB, the slurry was prepared by dissolving 200 g brown sugar in 250 ml water and then warming it for 15 minutes at 40°C. The slurry thus prepared was diluted 10 times with water and a packet of PSB culture obtained from the Microbiology Division, Indian Agricultural Research

Institute, New Delhi, was added to diluted slurry. The seeds were treated and then dried in shade for 4 h and sown. One bed was separately treated with inorganic fertilizers (Urea at 110 kg N ha^{-1}, superphosphate at 55 kg P ha^{-1} and murate of potash at 55 kg K ha^{-1}). Untreated beds and beds treated with inorganic fertilizers alone served as controls. The treatments were randomized with five replications. During the four-month growing period, necessary watering and weeding were done whenever required.

Plant-growth Parameters

After harvesting of *Trigonella foenum-graecum* crop, the plants were taken out along with the roots from each bed carefully and were gently rinsed with water to remove the soil. Fresh and dry weight of shoots and roots were taken separately and the number of pods per plant was counted. When the plants reached the flowering stage, the pollen fertility (%) was estimated by the method of Brown (1949) using satiability of pollen grains in 1% acetocarmine solution. The root-nodule index (on a 0-5 scale) was determined by visual observation, where 0= No nodulation, 1= Very light nodulation, 2= Light nodulation, 3= Moderate nodulation, 4= Heavy nodulation and 5= Very heavy nodulation. Nitrate reductase activity in leaves was estimated by the process of Jaworski (1971) and chlorophyll content of leaves was determined by the method of Hiscox and Israelstam (1979). One hundred milligrams of leaf pieces of chilli were placed in a vial containing 7 ml of dimethyl sulfoxide (DMSO) and the chlorophyll was extracted into the fluid by incubating for 60 minutes. The extract was transferred to a graduated tube and made up to 10 ml with DMSO and assayed immediately. A sample of 3 ml chlorophyll extract was transferred to a cuvette and the optical density (OD) values were measured at 645 and 663 nm in Spectrophotometer (Spectronic-1001) against a DMSO blank.

Nutrient Elements

The nutrient elements were studied in terms of N, P and K in soil as well as in plants. Nitrogen content in plants and residual soil nitrogen were determined according to the procedure given by IITA (1975). The phosphate and potash content from plants were estimated by the method of Lindner (1944). Similarly, phosphate and potash content in soil were determined by the method of Olsen *et al*. (1954) and Jackson (1973), respectively.

Statistical Analysis

The data of the two years were pooled and analyzed statistically according to Panse and Sukhatme (1978). Duncan's Multiple Range Test (DMRT) was employed to test for significant differences between the treatments.

RESULTS AND DISCUSSION

The data presented in Figure 1 clearly explained that soil application of various oil-seed cakes such as neem cake (NC) and castor cake (CC), botanicals like *Calotropis procera* (Cp) and phosphate-solubilizing bacteria (PSB) *Pseudomonas fluorescens,* singly as well as in various combinations, influenced growth parameters of fenugreek plant. Significant increase in growth was observed in all the treatments (T3-T13) as compared with beds treated with inorganic fertilizers (T2) and untreated control (T1). Highest improvement in growth parameters was observed with combined inoculation of NC+CC+Cp+PSB (T13).

Amongst inoculations, PSB (*Pseudomonas fluorescens*) alone significantly improved fresh as well as dry weight of plants, pollen fertility (%), number of pods plant-^{1}, nitrate reductase activity, and chlorophyll content over untreated control. This marked improvement could be due to pivotal role of PSB in solubilization of insoluble P through

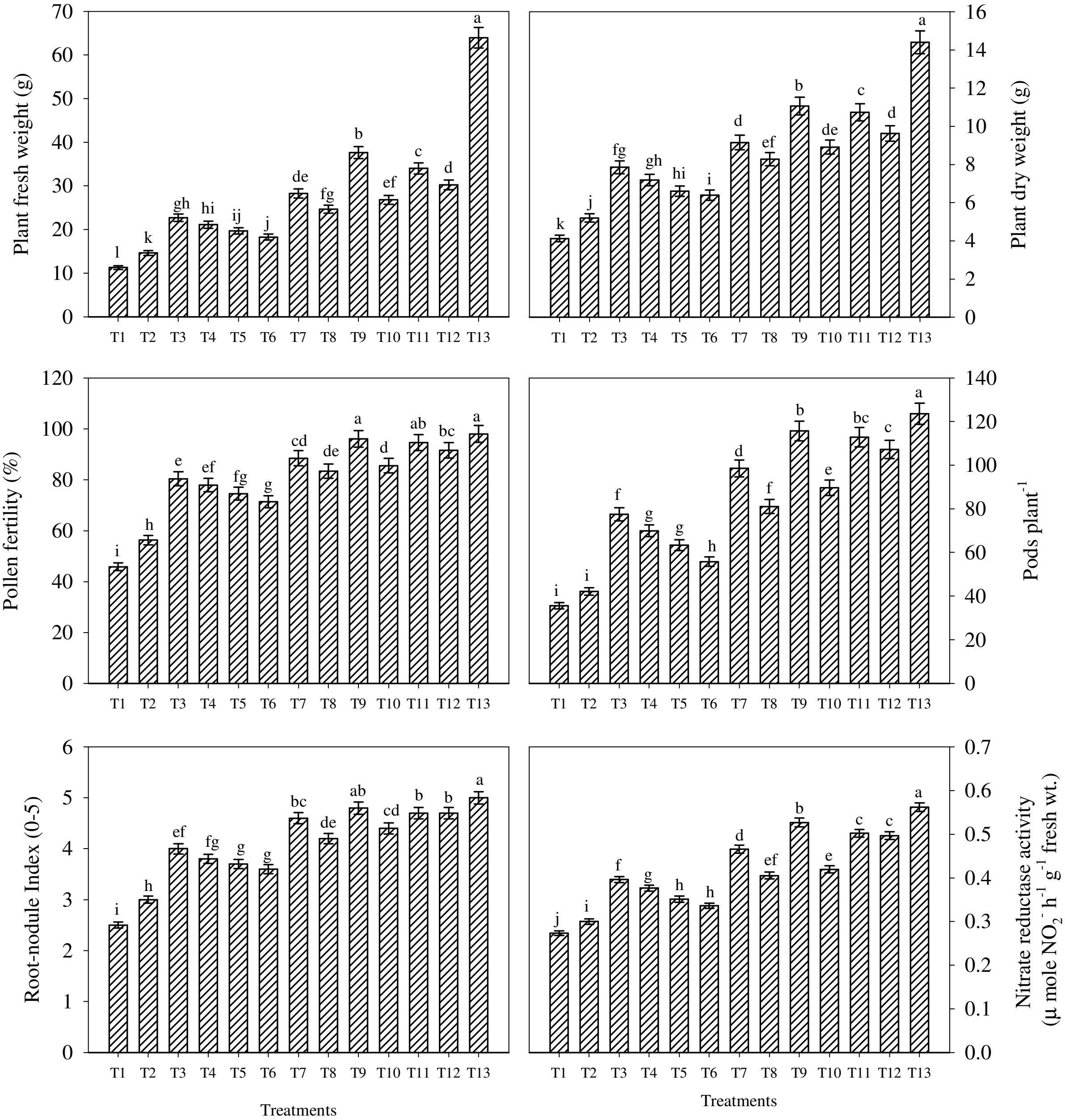

Figure 1. Effects of organic matters and phosphate solubilizing bacteria (PSB) singly and in various combinations on the growth of *Trigonella foenum-graecum*. Values are Mean±SE. Data labeled by the same letters did not differ significantly at $P< 0.05$. T1= Control; T2= Inorganic fertilizers; T3= Neem cake (NC); T4= Castor cake (CC); T5= *Calotropis procera* (Cp); T6= Phospahate-solubilizing bacteria (PSB); T7= NC+CC; T8= NC+Cp; T9= NC+PSB; T10= CC+Cp; T11= CC+PSB; T12= Cp+PSB, T13= NC+CC+Cp+PSB

production of organic acids. The adequate supply of P seems to have promoted root growth, resulting in higher production of root-nodule, thus better exploitation of N from soil as well as fixation of nodules and its reallocation in plant system (Bairwa, 2007). Our results are in accordance with Purbey and Sen (2005), Bhunia *et al*. (2006) and Rathore (2007).

Addition of organic matter has become very important in organic cropping system, which is increasing due to demand for chemical free products and due to the harmful effect of fertilizer on nodule formation. There are several reports which show that the combined and/or sole application of organic matters and biofertilizers increase yield and influence quality parameters in several crops (Worthington, 2001; Bahadur *et al*., 2006, 2003) and also show beneficial impacts in terms of physical (Kumar and Tripathi, 1990), chemical and biological properties of soil (Batra, 2004). Organic manures like oil-seed cakes supply micronutrients beneficial to the crop growth and productivity (Das *et al*., 2004). Organic matter acts as nutrient reservoirs and, upon decomposition, release organic acids, and the plants might absorb ions through their roots for the entire growth period, leading to higher yields (Kumar *et al*., 2009). Regular application of organics in amounts sufficient to meet the nutrient requirements of the crop not only results in increasing crop yield but also improves the soil fertility and organic matter content (Ramesh *et al*., 2006) and availability of plant nutrient as compared to chemical fertilizers (Brar *et al*., 2004). The positive effect of phosphate-solubilizing microorganisms may be due to better mobilization and supply of available phosphorus for crop growth and other yield attributes, since the phosphorus is associated with several vital functions of the plant such as utilization of sugar and starch, photosynthesis, and root growth. The chlorophyll content also increased in all the plants in those beds treated with oil-seed cakes, botanicals and PSB inoculated seeds (Figure 2). Biofertilizers not only provide nitrogen but also produce a variety of growth promoting substances (auxins, gibberellins) and vitamins (Hegde *et al*., 1999). The increased availability of N might lead to better root development as well as uptake and transportation of water and nutrients (Chandrikapore, 1999). The increased plant growth due to nutrients like N may be attributed to its role in enhancing chlorophyll content as nitrogen is one of the main components of chlorophyll, which increases synthesis of food material and their distribution towards the pods. Our results are in conformity with those of Jain *et al*. (2003), Sengupta *et al*. (2004) and Dubey *et al*. (2006). Integrated approach of nutrient supply through organic matters and biofertilizer like PSB gave better chlorophyll content. These observations are in agreement with those of Sharma and Thakur (2002).

A noticeable increase in root-nodulation was measured with the application of oil-seed cakes, botanicals and PSB, which might be due to rapid growth and proliferation and subsequently higher root-nodulation. Thus, phosphorus had a positive role in nodule development besides influencing photosynthesis. Our results are in accordance with the finding of Kasturikrishna and Ahlawat (2000), who reported that application of phosphorus significantly, increased the number of nodules and dry weight of nodules per plant. Gupta (2006) also observed that better nodulation in combined inoculation might be due to increased phosphorus availability through PSB and enhanced biological nitrogen fixation in green gram.

Significant increase in N, P and K content and uptake in plants and soil were estimated by increased fertility levels. The noticeable improvement in nutritional status of plants could be ascribed to the greater availability of nutrient in soil environment (Figure 2). Improvement in nutrient content could be attributed to the increased vegetative growth possibly as a result of effective utilization of nutrients absorbed through extensive root

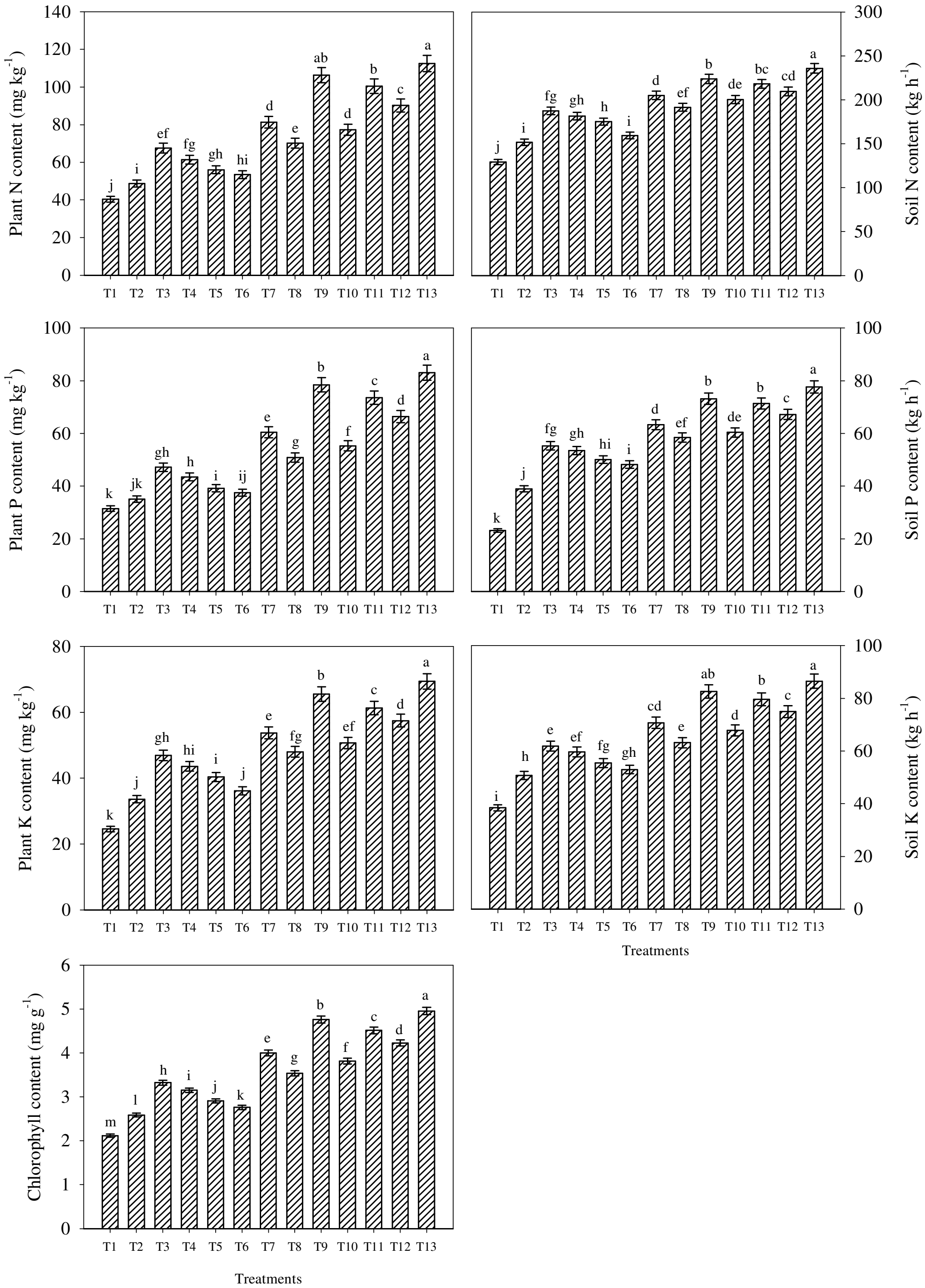

Figure 2. Effects of organic matters and phosphate solubilizing bacteria (PSB) singly and in various combinations on the chlorophyll content and productivity of *Trigonella foenum-graecum*. Values are Mean±SE. Data labeled by the same letters did not differ significantly at *P*< 0.05. T1= Control; T2= Inorganic fertilizers; T3= Neem cake (NC); T4= Castor cake (CC); T5= *Calotropis procera* (Cp); T6= Phospahate-solubilizing bacteria (PSB); T7= NC+CC; T8= NC+Cp; T9= NC+PSB; T10= CC+Cp; T11= CC+PSB; T12= Cp+PSB, T13= NC+CC+Cp+PSB

system developed under phosphate fertilization (Kumar and Kushwaha, 2006) and their efficient translocation towards sink components such as plant produce (Havlin *et al*., 2003). The nitrogen fertilization of fenugreek increases the cation exchange capacity of roots, enabling them to absorb more nitrogen from the soil, thus N and, subsequently, P might have been utilized in greater quantities due to their abundant availability (Nadeem *et al*., 2004). Potassium is considered as one of the nutrients which not only improves yield but also is involved in metabolic processes (Basseto *et al*., 2007; Imas and Magen, 2007).

The results of the present investigation clearly established that multi-inoculation improved growth of *Trigonella foenum-graecum* crop, soil fertility, and microbial activity in the soil and that the integration of organic matters and PSB may not only achieve highest yield and net return, but it may also increase soil fertility.

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نقش مواد آلی و باکتری های حل کننده فسفر(PSB) در رشد وعملکرد شنبلیله

ر. ریزوی، ی. محمود، س. ا. تیاگی

چکیده

در طی سالهای۲۰۱۱-۲۰۰۹، آزمایشی صحرایی در مزرعه تحقیقاتی دانشگاه اسلامی الیگار در هندوستان اجرا شد تا تاثیر بعضی کنجاله های دانه های روغنی شامل کنجاله گیاه نیم و کنجاله کرچک و گیاه دارویی *Calotropis procera*و کاربرد باکتری های حل کننده فسفات روی رشد و عملکرد(بهره وری) شنبلیله ارزیابی شود. این مواد به تنهایی یا به صورت ترکیبی در این آزمایش مطالعه شدند. پارامتر های رشد شامل وزن تر و خشک، باروری دانه ای گرده(٪)، تعداد نیام در گیاه، شاخص گره های ریشه، فعالیت ردوکتار نیترات، و مقدارکلروفیل بود. بهره وری بر حسب مقدار نیتروژن، فسفر، و پتاسیم در گیاه وخاک محاسبه شد. بر اساس نتایج، هرچند همه پارامترها به طور معنی داری در هر سه تیمار افزایش یافت، مصرف تکی و تنهای مواد در مقایسه با مصرف ترکیبی تاثیر کمتری داشت.در میان کنجاله های روغنی، کنجاله گیاه نیم در افزایش رشد گیاه بهتر از کنجاله کرچک بود و تاثیر مصرف *C. procera* و باکتری های حل کننده فسفر بعد از آن قرار داشت. گره بندی ریشه ها نیز در تیمار های ترکیبی افزایش چشمگیری نشان داد. مصرف ترکیبی کنجاله گیاه نیم، کنجاله کرچک، *C. procera* و باکتری های حل کننده فسفر در مقایسه با تیمارهای دیگر منجمله کار برد کود معدنی و تیمار شاهد بیشترین رشد و بهره وری را نشان داد.