Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 1197-1201
Received: 13-03-2019
Accepted: 15-04-2019

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Performance of chilli inoculated with arbuscular mycorrhiza and phosphate solubilizing bacteria under reduced fertilizer application

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Abstract
A field experiment was carried out at FOA Wadura Sopore, Kashmir to evaluate the performance of chilli inoculated with arbuscular mycorrhiza (AM) and phosphate solubilizing bacteria (PSB) when the fertilizer application was reduced to ½ and ¼ of the recommended fertilizer dose (RFD). The results of the study revealed that, inoculation of chilli with AM and PSB significantly increased all the parameters examined during the study. However, the best results were obtained when the seedlings were inoculated with AM followed by PSB at ½ of the RFD as evident from 16.6% and 12.6% increase in the total plant dry weight at the respective treatments as compared to control. Similarly the concentration of nitrogen (N), phosphorus (P), and potassium (K) in the plants inoculated with AM at ½ of the RFD was increased by 17.3, 20.6 and 23.2% followed by an increase of 2.5, 11.5, and 12.2% at PSB with ½ RFD as compared to control. Yield of chilli was respectively increased by 9.7% and 4.4 % when inoculated with AM and PSB at ½ of RFD as compared to control.

Keywords: Chilli, fertilizer, inoculation, PSB and AM

Introduction
Chilli (*Capsicum annuum* L.) is the universal spice and widely cultivated throughout temperate, tropical and subtropical countries. Titillating pungency and fascinating natural colour of chillies form an indispensable adjunct in every home all over the world. It is liked for its pungency, spicy taste besides the appealing colour it adds to the food. The ground powder and oleoresin are utilized in pharmaceutical preparations (Warrier, 1989) [29]. Chilli is rich source of vitamins A, C and E. Hundred gram of edible portion of capsicum provides 24 k cal of energy, 1.3 g of protein 4.3 g of carbohydrate and 0.3 g of fat. The principal coloring matter in chilli is a carotenoid pigment called as capsanthin, constituting about 35 per cent of the total pigment.

The chemical fertilizers like N, P and K have played a significant role in increasing the yield and quality of plant products during early seventies. However, indiscriminate use of chemical fertilizers has resulted in several problems like loss of fertility and multiple nutrient deficiencies. In addition to it, heavy inoculation of inorganic fertilizers degrade the soil health by adversely affecting the microbial biodiversity, physical and chemical environment of soil, water bodies and capital inputs like soil, water and thus overall ecology which ultimately result in reduced crop productivity and quality (Ghanti and Sharangi, 2009) [24]. The reason for the increased use of chemical fertilizers is the increased demand for food and the ability of some of the mineral elements to get fixed with soil colloids after application. For example P is an essential macro element for plants, yet the total concentration of P in soils ranges from 0.02-0.5%. Thus, to increase the availability of phosphorus for plants, large amount of fertilizers are used on a regular basis, because after inoculation, a large proportion of phosphorus is quickly transferred to an insoluble form (Omar, 1997) [18].

Keeping in view the adverse affects of application of inorganic fertilizers and their increasing cost, alternative technologies are to be developed through which the use of inorganic fertilizers can be minimized. In this regard the inoculation of microorganisms like AM and PSB seems to be an attractive solution that has been actively studied during the last decade so that dependence on inorganic fertilizers can be reduced as they have got the ability to supplement phosphorus, nitrogen, potassium, zinc etc to the plant. AM are widespread in nature and are fundamental component of the agro-ecosystem (Bethlenfalvay *et al.*, 1997) [5]. AM fungi are composed of fine, tubular filaments called hyphae. The mass of hyphae that forms the body of the fungus is called the mycelium. One of the most dramatic effects of infection by mycorrhizal fungi on the host plant is the increase in P uptake (Roger, 1991) [22] mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots (Asimi *et al*., 1980) [1]. The capacity of the root system to absorb nutrients is improved by the presence of external fungal hyphae that are much finer than plant roots and can reach beyond the areas of nutrient-depleted soil near the roots (Clarkson, 1985) [6]. Calculations show that a root associated with AM fungi can transport phosphate at a rate more than four times higher than that of a root not associated with AM fungi (Nye and Tinker, 1977) [17]. Similarly PSB are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds. P solubilization ability of rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Rashid *et al*., 2004) [20].

The application of biofertilizers has a significant effect on growth and yield of different vegetables. However, information on synergism between the different biofertilizers under reduced fertilizer application is still lacking. In this perspective it was thought worthwhile to carry out an experiment to find out the effect of AM fungus and PSB on different morphological, quality and yield parameters of under reduced inorganic fertilizer application.

Material and methods

A field experiment was carried out on Chilli at Faculty of Agriculture, Wadura Sopore (SKUAST-K). The soil of the experimental field is sandy loam with an EC of 0.33 µS/cm and pH 7.3.

Land preparation and fertilizer application

The experimental area was thoroughly prepared by ploughing three times and well decomposed farm yard manure (FYM) was applied at the rate of 25 t/ha followed by the preparation of plots. Fertilizers were applied to the plots as per the treatments as shown in table-1. N was applied to the plots in two doses, one at the time of transplanting and second in the month of June, whereas the entire P and K was applied in a single dose at the time of transplanting as per the designed treatments.

Treatments

Seven treatments shown in the table-1 were employed which were laid out in a randomized block design and replicated thrice. AM was applied in the root zone at the time of transplanting @ 10 Kg/ha, whereas seedlings were dipped in a solution of PSB for 45 minutes before transplanting.

Table 1: Treatment details

Symbol	Treatment
T0	Control
T1	½ RFD
T2	1/4RFD
T3	½ RFD + AM
T4	1/2RFD + PSB
T5	1/4RFD + AM
T6	1/4RFD + PSB

Intercultural operations

All the required cultural practices such as irrigation, weeding, pest and disease control etc. were kept constant and given uniformly in all the experimental plots. Ten plants were randomly selected from each plot in such a way that the marginal effect was avoided and data were recorded on the following parameters at the time of harvest.

1. **Plant height**: Plant height was measured with the help of a scale.
2. **Plant dry weight**: For calculation of plant dry weight, samples were oven dried at 80 ^{0}C till their weight became constant.
3. **Chlorophyll content**: Chlorophyll content was estimated at the time of second picking i.e. 85 DAT. The procedure developed by Witham *et al.* (1971) [30] was followed for chlorophyll estimation.
4. **Nitrogen (N):** N content from the bulbs was determined by using the method as proposed by Peoples *et al*. (1989) [19]
5. **Phosphorus (P)**: P content was estimated from the bulbs as per the method proposed by Koenig and Johnson (1942) [11].
6. **Potassium (K)**: K was estimated by flame photometeric method as proposed by Reed and Scott (1962) [21].
7. **Total soluble solids (TSS)**: TSS of the bulbs was measured by using the hand refractometer.
8. **Yield**: The ripe fruit yield obtained from each plot was recorded in all the pickings. The yield was calculated and expressed in tons per hectare.

Statistical analysis:

Data reported in this study are the mean values based on the three replicates. Differences among treatments were tested by ANOVA and the mean values among the treatments were compared using Duncan's multiple range test at p=0.05.

Results and Discussion

Plant height and total dry weight:

Plant height observed a continuous decrease when the fertilizer application was reduced to ½ and ¼ of the RFD. However, the inoculation with AM and PSB significantly increased the plant height as compared to control and the non inoculated plants which received a reduced fertilizer dose (table-2). Data indicates that the maximum increase in the plant height was observed in the plants inoculated with AM followed by PSB at ½ of the RFD. Height of the plants at these treatments respectively observed an increase of 8.4% and 1.9 % increase as compared to control. Similar to plant height, total plant dry weight also followed a decreasing trend with reduction in the fertilizer application to ½ and ¼ of the RFD. Total plant dry weight was significantly increased with inoculation of AM and PSB as evident from table-2. However, maximum increase of 16.6 % was observed by inoculating the plants with AM at ½ of the RFD followed by an increase of 12.6 % in the plants treated with PSB at the same level of the fertilizer dose as compared to control. The results are in agreement with Dar *et al*. (2018) [8] and Naik (2014) [15] who reported an increase in the plant height of onion through inoculation with AM and PSB at reduced phosphorus application which can be because of higher solubilization of P brought about by PSB followed by its increased uptake along with N and K as is evident from table-2 of this study. Inoculation with AM increases the absorbing capacity of the roots favoring more water uptake by the plants resulting in cell expansion responsible for the increased plant height (Huixing Song, 2005) [10]. It has been found that AM fungus improves the hydraulic conductivity and water uptake of roots hence providing a low resistance pathway for radial flow of water across cortex, and contributing towards better water uptake by the plants (Kothari *et al*., 1990) [12]. Furthermore, AM fungus may also regulate the hydraulic properties of plants through regulation of plasma membrane intrinsic proteins in combination with phytohormones (Ruiz-Lozano *et al*., 2009) [23]. Since nitrogen is a constituent of chlorophyll, the increase uptake of which might have resulted in increased synthesis of photosynthates leading to better plant growth. The second major nutrient phosphorus is an essential constituent of cellular protein and nucleic acid which might have encouraged the cell division and meristematic activity of plants resulting in increased plant height and total plant dry weight (Monika *et al*., 2006) [14].

Chlorophyll content:

Reduction in fertilizer application to ½ and ¼ of the RFD had an adverse affect on the plant chlorophyll content (table-2). However, inoculation with AM and PSB led to increase in the chlorophyll content at all the treatments. However, the maximum increase was observed in the plants subjected to inoculation with AM followed by PSB at ½ of the recommended fertilizer dose. Chlorophyll content at these treatments was respectively increased by 12.1% and 9.2% as compared to control.

The AM inoculated plants have shown to have higher chlorophyll content compared to non AM plants. This observation has been made by several workers in different systems (Auge, 2001) [2] and Colla *et al*. (2008) [6]. In pepper for instance, the chlorophyll content was shown to be increased by 15-25% to indicate the relevance of mycorrhizal association in increasing the pigment content in the leaves (Beltrano *et al*., 2013) [4]. It has been well recognized that chlorophyll concentration is related to photosynthetic rate and chlorophyll fluorescence. Thus, in AM inoculated plants an increased rate of chlorophyll has been associated with the increased rate of photosynthesis or with higher magnesium and nitrogen which are major constituents of chlorophyll (Mathur and Vyas, 1995) [13].

Effect on mineral uptake:

Mineral uptake observed a continuous decrease with the reduction in fertilizer application by ½ and ¼ of RFD. However, it was significantly improved with the inoculation of plants by AM and PSB. It is evident from table-3 that N content in the plant tissues was decreased by 55.2 and 68.4 % as compared to control when the RFD was reduced respectively by ½ and ¼. P concentration at ½ and ¼ of the RFD was decreased by 17.3 and 60.8 percent as compared to control followed by a reduction of 27.9 and 67.4% in K concentration in the plant tissues at the same treatments. Table-3 clearly indicates an increase in the concentration of N, P and K in the plants that were inoculated with AM and PSB. However the maximum increase was obtained in the AM inoculated plants treated with ½ of the RFD. N, P and K in the plant tissues treated with ½ of the RFD was increased by 17.3, 20.6 and 23.2 % as compared to control followed by an increase of 2.5, 11.5 and 12.2 % in the plants subjected to inoculation with PSB at ½ of the RFD. Similar results have also been found by Talwar *et al*. (2016) [26] and Hashem (2015) [9]. Microorganisms can increase the solubility of inorganic P by releasing protons, H^+ and organic acid anions such as citrate, malate and oxalate (Yousefi *et al*., 2011) [32] whereas the AM fungus absorbs the mineral elements like N, P, K, Fe, Zn etc much faster through physical exploration of the soil, modification of root environment, increased movement of various mineral elements into mycorrhizal fungus hyphae and their translocation to the associated plant (Turk *et al*., 2006) [28]. AM fungi expand the roots by adding their own expansive network of absorbing strands to mine the soil for water and the dissolved minerals carried therein may also alter the water relations and the drought tolerance of the plant. It has been found that AM fungus improves the hydraulic conductivity and water uptake of roots hence providing a low resistance pathway for radial flow of water across cortex, and contributing towards better water uptake by the plants (Kothari *et al*., 1990) [12]. Furthermore, AM fungus may also regulate the hydraulic properties of plants through regulation of plasma membrane intrinsic proteins in combination with phytohormones (Ruiz-Lozano *et al*., 2009) [24]. Tinker (1984) [27] while summarizing the effects of AM on increased water uptake indicated that the probable reason for increased water uptake are as the better distribution of absorbing hyphal network, greater surface area and better extension rate, altered soil rhizosphere properties, uptake kinetics, faster water extraction from reduced water potential soils and hence faster recovery from the water stress.

Total soluble solids

TSS observed a continuous decrease with the reduction in the RFD by ½ and ¼ as compared to control. Inoculation of the plants with AM and PSB increased the fruit TSS even at the reduced fertilizer application. However the maximum increase was observed at ½ of the RFD when plants were inoculated with AM followed by plants inoculated with PSB at the same fertilizer dose (table-3). Same results in this regard have been found by Talwar *et al*. (2017) [27] and Dar *et al*. (2018) [8] who found that inoculation with biofertilizers resulted in significantly higher TSS. The increase in TSS can be contributed to increased photosynthetic rate of the plants by inoculation with AM and PSB. It has been reported that microorganisms increase the chlorophyll content of the inoculated plants. In addition the stomatal conductance of the leaves is also accelerated by the microbial inoculation which favours rapid CO_2 diffusion into the leaves and both of these factors lead to increases in the photosynthetic rate of the plant (Mathur and Vyas, 2000) [13]. In addition more availability and uptake of K (table-3) which is having an important role in the translocation of photosynthates also might have contributed to increase TSS of onion by the combined inoculation with AM and PSB.

Yeild

The yield was severely reduced in chilli due to reduction in fertilizer application. The yield was reduced by 26.1 % and 50.7 % when the fertilizer application was reduced to ½ and ¼ of the RFD. Inoculation with AM and PSB significantly increased the yield even at the reduced fertilizer levels. However, AM fungus was found to have highest effect on the yield of chilli at ½ of the RFD where the yield was increased by 9.7% followed by an increase of 4.4 % at the same fertilizer dose and PSB inoculation as compared to control. This effect was in conformity with the results obtained by Subbiah (1994) [25] in chilli and onion, Nanthakumar and Veeraragavathatham (2000) [16] in brinjal and Yadav *et al*. (2005) [31] in onion. The significant increase in yield due to biofertilizers may be an account of its direct role in nitrogen fixation, production of phytohormone like substances and increased uptake of nutrients such as nitrogen. The increase in yield by the AM could be due to the function of mycorrhiza to enhance the efficiency of nutrient absorption and release of growth substances. This phenomenon of AM action might have increased the yield attributing characters in chilli as has been found by several workers *viz.*, Bagyaraj and Sreeramulu (1982) [3] in chillies, and Subbiah (1994) [25] in chilli and onion.

Table 2: Effect of reduced fertilizer application, arbuscular mycorrhiza and PSB on growth parameters and chlorophyll content of chilli

Treatments	Plant height (cm)	Plant dry weight (g)	Chlorophyll (mg g^{-1} FW)
Control	74.3	31.0	0.87
½ RFD	53.2	17.4	0.46
¼ RFD	34.5	11.2	0.23
1/2RFD + VAM	81.2	37.2	0.99
1/2RFD + PSB	75.8	35.5	0.92
1/4RFD + VAM	50.3	25.2	0.65
1/4RFD + PSB	42.4	23.2	0.53
CD (5%)	3.4	1.2	0.03

Table 3: Effect of reduced fertilizer application, arbuscular mycorrhiza and PSB on quality parameters and yield of chilli

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	TSS (%)	Yeild (t/ha)
Control	3.8	0.23	4.3	8.3	6.5
½ RFD	1.7	0.19	3.1	3.9	4.8
¼ RFD	1.2	0.09	1.4	1.6	3.2
1/2RFD +VAM	4.6	0.29	5.6	9.4	7.2
1/2RFD +PSB	3.9	0.26	4.9	8.5	6.8
1/4RFD +VAM	2.3	0.10	2.3	4.2	4.2
1/4RFD +PSB	1.9	0.15	1.9	3.9	3.7
CD (5%)	0.4	0.04	0.3	0.4	0.35

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