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Yield and economics of *Withania somnifera* influenced by dual inoculation of *Azotobacter chroococcum* and *Pseudomonas putida*

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Abstract: The yield parameters and cost economics of *Withania somnifera* were studied using dual inoculation of *Azotobacter chroococcum* and *Pseudomonas putida.* The experiment was conducted in a randomized block design with 3 replications for 2 consecutive years, along with 2 levels of organic manure, 10 t ha^{-1} and 20 t ha^{-1}. Inoculation with the inoculants generated encouraging results; root yield (1185.6 kg ha^{-1}), seed yield (208.13 kg ha^{-1}), number of offshoots per plant (6.07), and plant height (108.4 cm) were maximum with treatment T6 (organic manure [OM] 20 t ha^{-1} + both bacteria), followed by T9 (OM 10 t ha^{-1} + both bacteria), T4 (OM 20 t ha^{-1} + *A. chroococcum*), and T5 (OM 20 t ha^{-1} + *P. putida*), as compared to T1 (control), T2 (OM 10 t ha^{-1}), and T3 (OM 20 t ha^{-1}). The survival count of inoculated bacteria was highest 70 days after inoculation and declined thereafter. Economic analysis revealed that the net profit from treatment T6 was the highest with dual inoculation of bacteria and OM 20 t ha^{-1}, followed by OM 10 t ha^{-1} and dual inoculation of bacteria.

Key words: *Withania, A. chroococcum*, *P. putida*, yield, economics

Azotobacter chroococcum and *Pseudomonas putida* birlikte inokülasyonu ile etkilenmiş *Withania somnifera* ürün ve ekonomisi

Özet: Nitrojen bağlayabilen, fosfat ta çözünebilen ve bitkisel hormon üretebilen *Azotobacter chroococcum* ve *Pseudomonas putida* bakterilerinin biklikte inokülasyonunun *Withania somnifera*'nın ürün parametreleri ve ekonomik üretim gerçekleşmesi üzerine etkisi çalışılmıştır. Deney rastgele blok tasarımı ile birbirini takip eden iki yılda ve üç kez tekrar edilerek yapılmıştır. Organik atık miktarı 10 t ha^{-1} ve 20 t ha^{-1} olarak hesaplanmıştır. İnokulasyon sonuçları cesaret verici olmuştur. Kök verimi (1185,6 kg ha^{-1}), tohum verimi (208,13 kg ha^{-1}), bitki başına dallanma (6,07) ve bitki yüksekliği (108,4 cm) göz önüne alındığı zaman T1 (kontrol), T2 (OM 10 t ha^{-1}), ve T3 (OM 20 t ha^{-1}) kontrollerine göre T6 (organik atık (OM) 20 t ha^{-1} + iki tip bakteri) muamelesini takiben T9 (OM 10 t ha^{-1} + iki tip bakteri), T4 (OM 20 t ha^{-1} + *A. chroococcum*) ve T5 (OM 20 t ha^{-1} + *P. putida*) şeklinde muamele edilen bitkilerde en yüksek verim alınmıştır. İnoküle edilen bakterilerin yaşayan hücre sayımı ise 70 gün sonrası en yüksek bulunmuş daha sonra azalma gözlenmiştir. Ekonomik açıdan da T6 20 t ha^{-1} organik atıkta bakteri ekimini 10 t ha^{-1} organik manurlu bakterinin takibi şeklinde ekimli iki bakteri inokulasyonu ekonomik analizler sonucu en fazla verim alınan inokulasyon olarak bulunmuştur.

Anahtar sözcükler: *Withania, A. chroococcum*, *P. putida*, ürün, ekonomik

Introduction

Withania somnifera L. (Ashwagandha) belongs to family Solanaceae and is cultivated specifically for its root, which has medicinal and commercial value due the presence of the steroidal chemicals withanine and somniferine; therefore, it is considered the equivalent of Chinese ginseng (1). It has other beneficial uses, viz. as a diuretic, narcotic, cancer chemotherapy, and for radiosensitization (2). The main constituents of *Ashwagandha* are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. Due to these beneficial properties, *W. somnifera* is valued by consumers nationally and internationally (3). In India, cultivation of *W. somnifera* is gaining popularity among farmers; however, due to poor soil fertility, and costly chemical fertilizers and pesticides its production is not economical or profitable (4).

Biofertilizer has been used for a long time as an economic and sustainable input for increasing the productivity of a variety of crops, viz. vegetables (5), wheat (6-8), cotton (9), fodder crops (10), oil seed crops (11-13), and others (14).

Reports on the interaction of biofertilizers and *W. somnifera* are scanty; therefore, the present study was designed to determine the effect of the bacteria *A. chroococcum* and *P. putida*, along with 2 levels of organic manure (OM) (10 t and 20 t ha^{-1}) on yield and economic parameters of *W. somnifera.*

Materials and methods

The study was performed between October 2005 and March 2006 with *W. somnifera* var. Rakshita in a farmer's field in Meerut District, Uttar Pradesh, India. Available N, P, and K in the experimental plots were, respectively, 186, 4.2, and 268 kg ha^{-1}, with a pH of 7.7.

Treatments

T1: Control

T2: OM 10 t ha^{-1}

T3: OM 20 t ha^{-1}

T4: OM 20 t ha^{-1} + *A. chroococcum*

T5: OM 20 t ha^{-1} + *P. putida*

T6: OM 20 t ha^{-1} + *A. chroococcum* + *P. putida*

T7: OM 10 t ha^{-1} + *A. chroococcum*

T8: OM 10 t ha^{-1} + *P. putida*

T9: OM 10 t ha^{-1} + *A. chroococcum* + *P. putida*

Bacterial treatment of seeds

Bacterial cultures were obtained from the culture collection of Dr. S. Sharma, Department of Microbiology, SBS Post-Graduate Institute, Balawala, Dehradun, India. The seeds were treated with *A. chroococcum* and *P. putida*, separately and jointly. For treatment seeds were first sprinkled with a jaggery solution and then coated with charcoal containing respective bacterial cells (10^8 g^{-1}). The seeds were then dried for 30 min in shade and immediately sown in experimental plots. After 15 days of germination the plant population was maintained with 60 plants per meter square. Two levels of OM (10 t ha^{-1} and 20 t ha^{-1}) containing 0.75% N and 0.68% P were used in the experiment and it was obtained from a local market.

Survival studies

The rhizospheric soil that adhered closely to the roots was separated by gentle tapping and composite samples were prepared. Soil samples were air dried at room temperature and bacterial counts were made using a dilution technique on respective media (15); Azotobacter agar (Hi Media) for *A. chroococcum* and Kings B media (Hi-Media) for *P. putida*.

Studied parameters

Observations of root and seed yield (kg ha^{-1}), number of offshoots plant^{-1}, and plant height (cm) were recorded at the time of harvest (150 days after sowing). Bacterial survival was determined at regular intervals (35, 70, 105, and 150 days after inoculation). The data were analyzed at the 5% level of significance (16).

Results

In general, all quantitative plant traits increased significantly in response to OM. This response was enhanced further with bacterial inoculation (Table 1). All the treatments were superior to the control. The combined effect of biofertilizers and OM 20 t ha^{-1} (treatment T6) resulted in the highest root yield (1185.6 kg ha^{-1}), followed by T9 (1104.3 kg ha^{-1}). The

Table 1. Effect of biofertilizers and OM on *Withania somnifera* yield parameters.

Treatments	Root yield kg ha^{-1}	Seed yield kg ha^{-1}	Number of offshoots plant^{-1}	Plant height (cm)
T1	778.90	130.76	4.36	76.53
T2	885.06	148.16	4.55	84.36
T3	905.90	154.23	4.81	87.46
T4	1081.33	181.66	5.24	99.16
T5	1100.66	184.56	5.12	97.63
T6	1185.60	208.13	6.07	108.40
T7	1080.40	175.66	5.16	98.30
T8	1083.60	179.66	5.05	98.00
T9	1104.30	184.06	5.28	102.20
± SEM	9.53	3.30	0.086	0.338
CD at 5 %	28.58	9.91	0.258	1.013

number of offshoots plant^{-1} were maximum with T6 (6.07), T9 (5.28), T4 (5.24), T7 (5.16), and T5 (5.12). The control plot (T1) produced only 4.36 offshoots plant^{-1}, with minimum height (76.53 cm) and seed yield (130.76 kg ha^{-1}). The survival count of the inoculated bacteria was highest (*A. chroococcum* 3.2 × 10^4, *P. putida* 4.9 × 10^4) 70 days after inoculation and declined thereafter (0.9 × 10^4-3.6 × 10^4) and was minimum at the time of harvest (Table 2).

The economic analysis of the control treatment was calculated as follows: total cost (Rs. 15,450), gross return (Rs. 21,638), and net return (Rs. 6188). The benefit cost (B:C) ratio was determined to be 1.40 (Figures 1 and 2). The highest net return was with treatment T6, (Rs. 53,272 and a B:C ratio of 3.32), followed by treatment T9 for which the net return and B:C ratio were Rs. 44,763 and 2.94, respectively. Treatment T5 was good and the net return and B:C

Table 2. Survival count of *A. chroococcum* and *P. putida* at different time intervals (× 10^4).

Treatments	35 days		70 days		105 days		150 days	
	AC	PP	AC	PP	AC	PP	AC	PP
T1	0.8	1.1	1.7	2.0	1.0	1.4	0.9	1.2
T2	0.7	1.0	1.8	2.3	1.1	1.7	1.4	1.9
T3	1.2	1.3	1.9	2.2	1.4	2.0	1.7	1.6
T4	2.3	1.8	3.6	3.0	2.9	2.5	2.1	2.2
T5	1.6	2.7	2.2	4.8	2.0	3.6	1.7	3.6
T6	1.9	2.5	3.2	4.9	2.7	3.3	2.6	3.4
T7	2.0	2.0	3.5	3.3	3.7	2.8	2.2	2.8
T8	1.4	2.6	2.7	4.1	2.0	3.3	1.4	3.1
T9	1.8	2.1	3.1	4.5	2.3	3.4	2.3	3.0

AC: *Azotobacter chroococcum*; PP: *Pseudomonas putida*.

Each value is the mean of 3 replications.

ratio were Rs.3404 and 2.69, respectively (Figures 1 and 2).

Discussion

The present study aimed to evaluate the effect of inoculation with the bacteria *A. chroococcum* and *P. putida*, along with 2 levels of OM on yield and economic parameters of *W. somnifera*. In the present study it was evident that increases in OM increased growth parameters, which were enhanced further by the application of bacteria (5,6). The rhizosphere of different crops harbor several types of microbes and the productiveness of the rhizosphere for the inoculated bacteria might be attributable to the favorable influence exerted by root exudates (8,9,12), which contain amino acids, carbohydrates, organic acids, and plant growth substances.

It is evident from Table 1 that treatment with single or dual bacteria, along with OM resulted in higher plant yield parameters, as compared to treatments T1, T2, and T3, which did not include bacterial inoculation. Table 1 shows that root yield was significantly higher in all treatments, as compared to the control (13.62%-52.21%); similarly, seed yield (13.3%-59.16%) was higher than that of the control. Inoculation with both bacteria and OM 20 t ha^{-1} resulted in 30.87% higher root yield, as compared to T3 (OM 20 t ha^{-1}). Similarly, dual inoculation (T8) resulted in 24.77% higher yield than that of T2 (OM 10 t ha^{-1}). Dual inoculation of bacteria with OM 20 t ha^{-1} resulted in higher seed yield (34.94%) in T6 than in T3, and T9 produced 24.23% more seed yield than T2. The increase in T6 and T9, as compared to T3 and T2, respectively, was due to the application of beneficial microbes, as the OM level in all 4 treatments was the same (20 t ha$^{-1)}$. Treatments T4 and T5 were superior to T3 (OM 20 t ha^{-1}), while T7 and T8 were superior to T2 (OM 10 t ha^{-1}). The bacterial survival count also increased steadily and reached a maximum 70 days after sowing, which was due to the gradual and regular release of root exudates, resulting in survival and proliferation of bacteria (Table 2). At later stages root exudates decreased due to the shift of nutrient flow to the sink and, therefore, a decline in the population of bacteria (17). The stimulatory effect of OM on the survival of *A. chroococcum* and *P. putida* might have been exerted directly through its effect on the growth and proliferation of the bacteria, thereby creating a favorable habitat for better survival of the inoculated bacteria (6,18).

Economic analysis revealed higher profitability in the presence of both bacteria. It is evident from Figures 1 and 2 that treatment T6, followed by treatment T9, were more economically profitable, as net return was higher compared to the other treatments.

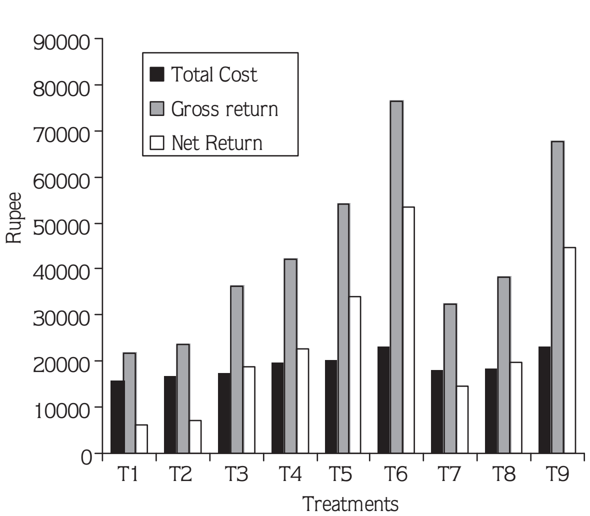

Figure 1. Economic analysis of the treatments.

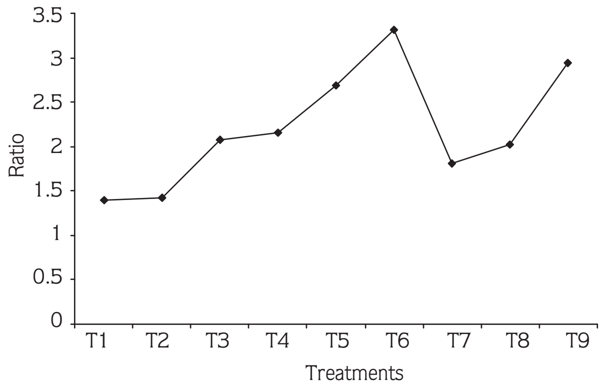

Figure 2. Cost–benefit ratio analysis of the treatments.

Treatments T3, T4, T5, and T8 also showed good results in the presence of the microorganisms. Our results are in agreement with those reported by Kumar et al. (7), who also calculated the economic efficacy of tall and dwarf wheat varieties with phosphate solubilizing *A. chroococcum* mutants and parent strains; they calculated a gain of Rs. 2.87-31.28 ha^{-1} by investing 1 Indian Rupee ha^{-1}. Our study suggests that microbial inoculants can be used as an economical input to increase crop productivity, to maintain soil sustainability, and to harvest more nutrients.

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