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Effect of Some Biological Agents and Naphthalenacetic Acid (NAA) on Rooting Response of Some Ornamental Shrubs

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Abstract. The effect of Naphthalenacetic acid (NAA), *Pseudomonas putida* and *Trichoderma harzianum* on the rooting response of *Clerodendron inerme*, *Bougainvillea spectabilis* and *Nerium oleander* was studied.

The data indicated that the percentage of rooted cuttings was affected by the different treatments and the different plant types. NAA, *P. putida* and *T. harzianum* increased the percentage of rooting of *C. inerme*, *B. spectabilis* and *N. oleander*. Although all treatments increased the rootings of all tested plants, *P. putida* showed significant increase over other treatments with cuttings of *B. spectabilis*.

The number of leaves of *B. spectabilis* was increased as a result of NAA, *P. putida* and *T. harzianum* treatments. However, the number of *C. inerme* leaves was increased with NAA and *T. harzianum* treatments. In contrast, the number of leaves of *N. oleander* was not affected by any of the studied treatments. No significant differences were detected in leaves dry weight of the treated plants.

The length of branches of *B. spectabilis* was encouraged with NAA, *P. putida* and *T. harzianum* treatments. However, only *P. putida* increased the length of *N. oleander* branches.

The root length of the treated *C. inerme* with NAA, *P. putida* and *T. harzianum* was increased. The root dry weight of *C. inerme* was increased with NAA and *P. putida* treatments, whereas, no increase in both of root length and dry weight of *B. spectabilis* and *N. oleander* was observed.

Introduction

Auxin treatment is known to promote root formation on cuttings of several plant species. Stevens [1] found that three thousand parts per million of Indolbutyeric acid (IBA) improved root index of *Praceana merginta*. Gupta and Kher [2] treated the terminal cuttings *Tabernaemontana coronaria* with IAA, IBA or NAA, each at 2000, 4000 or 6000 ppm. They reported that the highest rooting (86.66%) and survival (92.30) were obtained with IBA at 2000 ppm.

In 1989, Gupta and Kher [3] found that the optimum treatment for maximum rooting and survival of *Dombeya natalensis* was recorded with IBA at 4000 ppm. This treatment also gave a greater number of roots and more root length per cutting than treatments with IAA or NAA.

The effect of the different rooting hormone on the rooting percentage varied widely according to species and treatments [4]. Kol'Tsov [5] in three year rooting trials treated semi-woody cuttings from *Corylus colrna* taken in summer with several substances including IBA, IAA, succinic acid+ papain, and CCC (Chlormequat). He found that the best results were obtained by IBA at 0.005 –0.01%.

The biological agents are generally applied to control several pathogenic agents such as *Fusarium avenaceum, F. oxysporum* and *Rhizoctonia solani* causing diseases to field crops, ornamentals, shrubs and trees especially during the first phase of plant growth and cutting propagations [6, 7, 8, 9]. However, plant growth was increased as a result of using some useful biological agents [6, 10, 11]. Moreover, few investigations studied the effect of the biological control agents on plant cuttings [6, 12], and reported that the rooting percentage was increased.

The objective of this work was to investigate the effect of using a rooting hormone (NAA) and the biological agents *Pseudomonas putida* and *Trichoderma harzianum* on the rooting response of cuttings of some ornamental shrubs (*Clerodendron inerme, Bougainvillea spectabilis* and *Nerium oleander*).

Materials and Methods

This study was conducted in the greenhouse of the Horticulture Department, College of Agriculture and Food Science, King Faisal University during the end of Feb. 1992. Wooden cuttings (15 cm length) were collected from the following ornamental shrubs: *C. inerme, B. spectabilis* and *N. oleander*. Naphthalenacetic acid (NAA at 40%); *P. putida* and *T. harzianum* were used in this study.

P. putida was isolated from soil suppressive to *F. oxysporum* a pathogen causing wilt disease. The culture was rehydrated and grown in flask containing 50 ml King B (KB) broth on a shaker for 24 hr at 25 ± 1 C°. The cells were centrifuged at 2500 g for 10 min., rinsed and resuspended in a solution of 0.1 M $MgSO_4$. $MgSO_4$ was used to prevent osmotic bursting of the bacteria. The concentration of the bacteria suspension was determined at 780 nm. According to Scher and Baker [8] an optical density of 0.9 yielded a bacterial suspension containing 10^9 colony forming unit per ml (cfu/ml). The wounded basal end of the cutting was sprayed with the suspension containing *P. putida* at 10^9 cfu/ml before planting in the rooting media.

T. harzianum was isolated from soil suppressive to *R. solani* causing damping off disease for several host plants) according to the method of Chet and Baker [9]. A conidia of *T. harzianum* was grown on yeast-malt extract agar medium consisting of 2g yeast extract, 20g malt extract, and 20g agar per liter of distilled water. Conidia were then removed from the surface of the culture with sterilized water, and adjusted, by use of a hemacytometer, to 10^6 conidia per ml. This suspension in water was placed in sprayer and directly applied to basal end of plant just before planting in the rooting media.

Biological agents used in this work were isolated and propagated in the Plant Protection Department, College of Agricultural and Food Sciences, King Faisal University.

Four treatments were conducted for each plant; control (untreated cuttings), NAA 40%, *P. putida* and *T. harzianum.* For each treatment, thirty cuttings from each plant species were collected and planted in three plastic pots (20 cm diameter) using a mixture of sandy soil and peatmoss at the rate of (1:1 v/v). In each pot 10 cuttings were planted. The treated cuttings and the untreated ones were replicated three times. The pots were placed in a controlled greenhouse (20±5 °C). The experiment was designed as a completely randomized block. After 60 days from planting, the following data were recorded: percentage of rooted cuttings, number of leaves/plant, number of branches/plant, length of roots (cm) and dry weight of leaves and roots (gm). Data were subjected to statistical analysis according to Snedecor and Cochran [13].

Results and Discussion

Data of Table 1 indicated that the percentage of rooted cuttings of *C. inerme* was significantly affected by the different treatments. The untreated plants demonstrated the lowest percentage (36.6%). However, the rooting percentages were 73.3, 80.0 and 80.0% in cuttings treated with NAA, *P. putida* and *T. harzianum,* respectively. No significant differences were observed between NAA and the biological agents treatments. Similar growth responses were reported with several hosts [6, 11, 12] and auxins [2, 3, 5].

Table 1. Effect of NAA; *Pseudomonas putida* and *Trichoderma harzianum* on rootings percentage, number of leaves, length of branches and dry weight of leaves and roots of *Clerodendron inerme*

Treatments	Rooting percentage	Number of leaves	Length of branches (cm)	Length of roots (cm)	Leaves dry weight (g)	Roots dry weight (g)
Control	36.60[a]	07.13[a]	17.93	2.53[a]	0.20	0.12[a]
NAA	73.30[b]	12.91[b]	18.53	9.83[b]	0.33	0.22[b]
P. putida	80.00[b]	09.66[a]	19.73	9.50[b]	0.40	0.21[b]
T. harzianum	80.00[b]	13.83[b]	21.20	7.93[b]	0.33	0.11[a]
L.S.D. ($P = 0.05$)	27.7	2.59	NS	2.40	NS	0.02

The number of leaves per cutting in the control as well as the *P. putida* treatments was nearly the same and was significantly lower than the NAA and *T. harzianum* treatments which had an average of 12.9 and 13.8 leaves, respectively.

No significant differences were detected between the different treatments for both the length of branches and the dry weight of leaves. The root length showed the same trend as the percentage of rooted cuttings, where it was significantly increased by any of the applied treatments. However, root dry weight was significantly increased only by the NAA, and *P. putida* treatments.

The obtained data in Table 2 revealed that the cuttings of *B. spectabilis* treated with NAA or *P. putida* or *T. harzianum* showed a highly significant increase in percentage of rooted cuttings. The highest rooting percentage was demonstrated from cuttings treated with *P. putida,* which was significantly higher than the NAA and *T. harzianum* treatments. The number of leaves per cutting was signficantly increased by all treatments, with NAA treatment being the most effective. Its average was 28 leaves/plant, which was significantly higher than the other two treatments.

Regarding the length of branches, data was significantly increased by the NAA (20.63 cm) and *P. putida* (26.0 cm) treatments as compared to the control (10.8 cm). The difference between the control and *T. harzianum* treatment on one hand, or between the later and the NAA or the *P. putida* treatments were not significant. Dry weight of leaves and roots and the length of roots showed no significant effects of the treatments on these characters when compared to the untreated cuttings.

Table 2. Effect of NAA; *Pseudomonas putida* and *Trichoderma harzianum* on rootings percentage, number of leaves, length of branches and dry weight of leaves and roots of *Bougainvillea spectabilis*

Treatments	Rooting percentage	Number of leaves	Length of branches (cm)	Length of roots (cm)	Leaves dry weight (g)	Roots dry weight (g)
Control	36.66^{a}	10.40^{a}	10.86^{a}	08.83	0.23	0.17
NAA	63.33^{b}	28.00^{c}	20.63^{b}	13.20	0.70	0.26
P. putida	83.33^{c}	20.33^{b}	26.00^{b}	10.36	0.53	0.26
T. harzianum	66.66^{b}	18.03^{b}	19.23^{ab}	12.10	0.56	0.36
L.S.D. ($P = 0.05$)	19.59	4.08	9.09	NS	NS	NS

The data in Table 3 indicated that treated cuttings of *N. oleander* with NAA *P. putida* and *T. harzianum* demonstrated significant increase in the rooting percentage as compared to the untreated cuttings. The highest rooting percentage was obtained from cuttings treated with the biological agents. However, no significant differences

Table 3. Effect of NAA; *Pseudomonas putida* and *Trichoderma harzianum* treatments on rootings percentage, number of leaves, length of branches and dry weight of leaves and roots of *Nerium oleander*

Treatments	Rooting percentage	Number of leaves	Length of branches (cm)	Length of roots (cm)	Leaves dry weight (g)	Roots dry weight (g)
Control	50.00[a]	24.80	16.43[a]	10.96	0.50	0.13
NAA	73.30[b]	28.30	16.80[a]	11.10	0.63	0.23
P. putida	83.30[b]	25.80	20.80[b]	11.33	0.66	0.16
T. harzianum	83.30[b]	26.90	18.30[ab]	13.26	0.70	0.20
L.S.D. ($P = 0.05$)	23.06	NS	2.81	NS	NS	NS

were obtained in rooting percentages of cuttings treated with NAA and either *P. putida* or *T. harzianum*. The different treatments had no significant effect on the number of leaves, the dry weight of leaves and roots as well as the length of roots. However, the length of branches was significantly increased in the *P. putida* treatment (20.8 cm).

The response of rooting hormone (NAA) and biological agents on the rooting percentage is known to differ according to plant species (Fig. 1). This result is in agreement with that of Kralik and Psota [4].

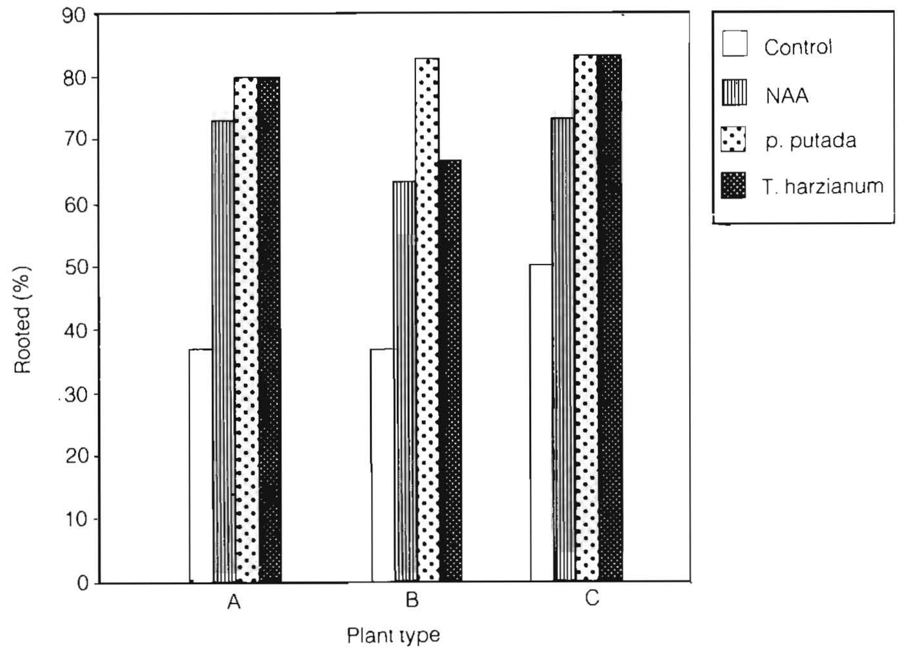

Fig. 1. Effect of NAA, *Pseudomonas putida* and *Trichoderma harzianum* on rooting percentage of *Clerodendron inerme* (A), *Bougainvillea spectabilis* (B) and *Nerium oleander* (C).

It should be pointed out that the biological agents subjected in the present study are live microorganisms where they grow and reproduce under suitable conditions. Thus the application of such biological agents to the cuttings introduced them to the soil media. Planting cuttings in soil media infested with such biological agents may generate the beneficial effects mentioned in this report. Therefore, the use of biological agents might be recommended in this sense and preferred over the use of rooting hormone since it would be much less in cost.

In conclusion the biological agents were the most effective factors for promoting the highest rooting percentages, although the response of the biological agents was significantly similar to the rooting hromone treatments.

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تأثير بعض الكائنات المستخدمة في المكافحة الحيوية وهرمون نفثلين أسيتك أسيد على الاستجابة للتجذير لبعض شجيرات الزينة

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ملخص البحث. أجريت دراسة لمعرفة تأثير استخدام هرمون نفثلين أستيك أسيد والكائنات الحية والمستخدمة في مكافحة الأمراض النباتية *Pseudomonas putida* و *Trichoderma harzianum* على الاستجابة للتجذير لعقل نباتات الزينة *Clerodendron inerme* و *Bougainvillea spectabilis* و *Nerium oleander*. أظهرت النتائج أن نسبة التجذير تتأثر باختلاف المعاملات وباختلاف نوع النبات. حيث أدت المعاملة بـ *T. harzianum;* NAA و *P. putida* لزيادة نسبة التجذير في جميع النباتات المختبرة بالمقارنة بالنباتات غير المعاملة. وعلى الرغم من أن جميع المعاملات أدت إلى زيادة التجذير إلا أن المعاملة *P. putida* أدت إلى زيادة معنوية على جميع المعاملات في نبات *B. spectabilis*.

كما أظهرت النتائج زيادة في عدد الأوراق لنبات *B. spectabilis* عند المعاملة بـ: NAA؛ *P. putida* و *T. harzianum* بينما زاد عدد أوراق النباتات لنبات *C. inerme* عند المعاملة بـ: NAA و *T. harzianum* فقط. ولم تظهر النتائج زيادة في عدد الأوراق لنبات *N. oleander*. أو زيادة في الوزن الجاف في جميع النباتات المختبرة.

لوحظ أن المعاملة بـ: NAA ؛ *P. putida* و *T. harzianum* تشجع الزيادة في طول الأفرع في عقل نباتات *B. spectabilis*. بينما شجعت المعاملة بـ: *P. putida* طول الأفرع في عقل *N. oleander*. ولم يلاحظ زيادة في عدد الأفرع في جميع المعاملات لنبات C. inerme.

معاملة النباتات بـ: NAA ؛ *P.putida* و *T. harzianum* أدت إلى زيادة في طول الجذور لنبات *C. Nemreni* بينما أظهرت المعاملة بـ: NAA و *P. putida* زيادة في الوزن الجاف للجذور. ولم تظهر جميع المعاملات زيادة معنوية في أطوال جذور كل من *B. spectabilis* و *N. oleander*.