Effect of Bio Control Agents (*Trichoderma viride* and *Pseudomonas fluorescense*) on Rooting of Pomegranate (*Punica granatum* L.) Cutting

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**Abstract**

The present experiment entitled Effects of bio control agents (*Trichoderma viride* and *Pseudomonas fluorescense*) on rooting of pomegranate (*Punica granatum* L.) cuttings was conducted at nursery, Horticulture Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), during the period of 2018-19. The present experiment was conducted to study the effect of bio control agents and water as control (viz., control, *Trichoderma viride* @ 5% rooting media, *Pseudomonas fluorescense* @ 5% rooting media ) with three replication in a complete randomized design (CRD), under shade net condition. The study revealed that significant differences were existed among the treatments for different rooting and shooting parameters. Among the different treatments the earliest sprouting of cutting as well as significantly highest percentage of success, number of leaves per shoot, length of root, diameter of root, fresh weight of roots, and number of roots per cutting were observed in *Trichoderma viride* @ 5% rooting media followed by *Pseudomonas fluorescense* @ 5% rooting media. However the performance of cutting in treatment control was inferior.

**Keywords**
Pomegranate, *Trichoderma viride*, *Pseudomonas fluorescense*, Bio control agents, *Punica granatum*

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**Introduction**

Pomegranate (*Punica granatum* L.) belonging to family Lythraceae, is an ancient fruit originated in Persia, Afghanistan and Baluchistan (De Candolle, 1967). Pomegranate is important crop of India and is said to be native to Iran (Persia) have some special botanical characteristics, the tree have a identical bushy shape having multiple-stems, the bushiness in plant is because of suckers routinely arising from the base. The plant has an average height of 5-8 m tall. The plant is normally deciduous in nature. The newly arrived shoots are thin and weepy caring thorns. The color of the leaves is dark green with a shiny appearance and the size of the leaves is small with alternate arrangement.

The plant is monoecious with two types of conspicuous flowers which arise in the new grown stems in the spring season, major bloom period is the spring season. The nutritive value of pomegranate fruits is very high and has several health benefits. Pomegranate fruits are rich in vitamin C,
potassium and antioxidants. Nutritional value of 100g of edible arils is having 346KJ energy, 18.7 g carbohydrates, 13.7 g sugars, 1.7 g protein, 1.2 g fat, 236 mg potassium, 10 mg vitamin C, 0.07 mg thiamine and 4.0 g dietary fibre. The fruits also have therapeutic values accompanying considerable pharmacological properties like antimicrobial, antiviral and antimutagenic effects (Negi et al., 2003; Seeram et al., 2005).

Pomegranate is propagated by both sexual and asexual means. Rhizogenesis is the most habitually used organogenetic phenomenon in vegetative multiplication of pomegranate. Pomegranates can be propagated using both softwood or hardwood cuttings, but hardwood cuttings are commercially adopted methods.

Certain microorganism like *Trichoderma viride*, and *Pseudomonas* also found to induce roots in pomegranate by suppressing the attacks of several disease causing pathogens and reducing biotic stress at the root zone. Further nowadays organic pomegranates production requires the cutting which are propagated by utilization of organic natural products, which can be done by using organic compost and biocontrol agents.

**Materials and Methods**

The present investigation was carried out from September 2018 to February 2019 at nursery Horticulture Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). The type of cuttings used was hard wood cuttings of uniform size (15-20 cm long) with 5-6 functional buds were taken from vigorous, healthy plants of pomegranate variety Super Bhagva. For the preparation of cutting healthy, vigorous, disease free plant of pomegranate variety Super bhagva was selected. The partially matured branches, 0.75-1.00 cm in thickness were taken for cuttings preparation. The cuttings of 15-20 cm in length with 5-6 functional buds were prepared for planting and the leaves removed entirely. Shortly after their preparation, cuttings were kept in water to maintain the amount of moisture until planting time.

Whereas, Bio control agents @ 5% were mixed as per treatment in the rooting medium. Two third parts of the treated cuttings were placed in the rooting media at a slight angle (about 60°) vertical to the plane. The rooting media was provided water to supply moisture to the cutting and soil around the cutting area was pressed lightly to fix the cutting in rooting media.

**Results and Discussion**

**Days taken to start sprouting of cuttings**

Data presented in table 1 shows that there was a significance difference present between the treatments, for the days taken to start sprouting and the days taken for the initiation of sprouting of cuttings ranged from 7.67 days to 12.00 days. The earliest sprouting of cutting was recorded in *Trichoderma viride* @ 5% of rooting media (7.67 days), followed by *Pseudomonas fluorescence* @ 5% of rooting media (8.0 days). Whereas, late sprouting of cuttings (12.00 days) were recorded under control. Studies show that soil fungi *Trichoderma viride* produced considerable amount of auxins, which is essencial for initiation of sprouting in cuttings (Manka et al., 1997) (Fig. 1).

**Days taken to 50% sprouting of cuttings**

The data presented in table 1 shows that the days taken to 50% sprouting of cutting ranged from 26 to 30.67 days. The minimum days taken to 50% sprouting of cuttings was observed under *Trichoderma viride* @ 5% of rooting media was (26 days), followed by
Pseudomonas fluorescence @ 5% of rooting media (27.67 days). Whereas, maximum days taken to 50% sprouting of cuttings (30.67 days) were recorded under control.

**Percentage of success of cuttings**

The data presented in table 1 show that the percentage of success of cuttings ranged from 66.67 to 83.33%. The maximum percentage of success of cutting was observed under Trichoderma viride @ 5% of rooting media (83.33%), followed by Pseudomonas fluorescence @ 5% of rooting media (80%). Whereas, minimum percentage (66.67%) of success of cuttings was recorded under control. The result obtained is in harmony with the results of Jaganath *et al.*, (2009).

**Number of shoots per cutting**

The data presented in the table 2 at 30 DAP shows that the number of shoots per cutting ranged from 3.27 to 5.73. The maximum number of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (5.73). Whereas, minimum numbers of shoots per cutting were observed under Control (3.27). At 60 DAP the number of shoots per cutting ranged from 4.20 to 6.13.

The maximum number of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (6.13). Whereas, minimum numbers of shoots per cutting were observed under Control (4.20). At 90 DAP the number of shoots per cutting ranged from 4.67 to 6.73. The maximum number of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (6.73). Whereas, minimum number of shoots per cutting were observed under Control (4.67). At 120 DAP the number of shoots per cutting ranged from 5.47 to 7.60. The maximum number of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (7.60). Whereas, minimum number of shoots per cutting were observed under Control (5.47).

At 150DAP the number of shoots per cutting ranged from 6.00 to 8.40. The maximum number of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (8.40). Whereas, minimum number of shoots per cutting was observed under Control (6.00). At 180DAP the number of shoots per cutting ranged from 6.80 to 9.07. The maximum number of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (9.07). Whereas, minimum numbers of shoots per cutting were observed under Control (6.80) (Fig. 2).

**Length of shoots (cm)**

Data presented in the table 3 shows that, at 30 DAP the length of the longest shoots per cutting ranged from 8.41 to 12.07. The maximum length of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (12.07). Whereas, minimum length of shoots per cutting were observed under Control (8.40). At 60, 90, 120, 150, 180 DAP the length of shoots per cutting ranged from 15.09 to 21.77, 20.47 to 25.80, 23.27 to 29.40, 25.80 to 30.75, 27.79 to 34.79 respectively.

The maximum length of shoots per cutting was observed under Trichoderma viride @ 5% of rooting media (21.77, 25.80, 29.40, 30.75, 34.79, respectively). Whereas, minimum length of shoots per cutting were observed under Control (15.09, 20.47, 23.27, 25.80, 27.79, respectively). Trichoderma is responsible for increasing the length of shoots by providing a healthy root system and increasing the level of auxin. The results are in line with the findings of Patil *et al.*, (2001) in pomegranate (Fig. 3).
Number of leaves per shoot

Data in table 4 shows that, at 30DAP the number of leaves per shoots ranged from 8.73 to 12.53. The maximum number of shoots per cutting was observed under *Trichoderma viride* @ 5% of rooting media (12.53) T9 followed by *Pseudomonas fluorescence* @ 5% of rooting media (11.40). Whereas, minimum number of leaves per shoots were observed under Control (8.40).

At 60, 90, 120, 150 and 180 DAP the number of leaves on selected shoots ranged from 14.33 to 19.00, 20.67 to 25.00, 24.93 to 28.87, 27.40 to 31.13 and 29.87 to 33.67 respectively. The maximum number of leaves per shoot was observed *Trichoderma viride* @ 5% of rooting media (19.00, 25.00, 28.87, 31.13 and 33.67 respectively). Whereas, minimum number of leaves per cutting were observed under control (14.33, 20.27, 24.93, 27.40 and 29.87 respectively) (Fig. 4).

Total number of leaves per cutting

The data in table 5 shows that, at 30DAP the total number of leaves per cutting ranged from 31.40 to 40.00. The maximum number of leaves per cutting was observed under *Trichoderma viride* @ 5% of rooting media (40.00). Whereas, minimum number of leaves per cutting were observed under Control (31.40).

At 60 DAP the total number of leaves per cutting ranged from 65.33 to 78.93. The maximum number of leaves per cutting was observed under *Trichoderma viride* @ 5% of rooting media (78.93). Whereas, minimum number of leaves per cutting were observed under Control (65.33).

At 90, 120, 150 and 180 DAP the total number of leaves per cuttings ranged from 75.33 to 89.60, 83.60 to 94.67, 86.67 to 98.73 and 89.40 to 100.40 respectively. The maximum number of leaves per cutting was observed *Trichoderma viride* @ 5% of rooting media (89.60, 94.67, 98.73 and 100.40). Whereas, minimum number of leaves per cutting were observed under Control (75.33, 83.60, 86.6 and 89.40) respectively (Fig. 5).

Survival percentage of cuttings

The data presented in table 6 shows that the survival percentage of cuttings ranged from 56.67 to 76.67 %. The maximum survival percentage of cutting was observed under *Trichoderma viride* @ 5% of rooting media (76.67%). Whereas, minimum percentage (56.67%) of success of cuttings was recorded under control.

Root characters

Number of roots per cutting

The data presented in table 6 shows that the number of roots per cutting ranged from 25.27 to 36.47. The maximum number of roots per cutting was observed under *Trichoderma viride* @ 5% of rooting media (36.47). Whereas, minimum percentage (56.67%) of success of cuttings was recorded under control.

Length of roots (cm)

The data presented in table 6 shows that the length of roots ranged from 20.96 to 28.37. The maximum length of roots was observed under *Trichoderma viride* @ 5% of rooting media (28.37). Whereas, minimum length of roots (20.96) was recorded under control.

Diameter of roots (mm)

The data presented in table 7 shows that the diameter of roots ranged from to 0.73 to 1.73.
The maximum diameter of roots was observed under *Trichoderma viride* @ 5% of rooting media (1.73). Whereas, minimum diameter of roots (0.73) was recorded under control (Fig. 7a).

**Fresh weight of roots (g)**

The data presented in table 7 shows that the fresh weight of roots ranged from 0.81 to 1.69. The maximum fresh weight of roots was observed under *Trichoderma viride* @ 5% of rooting media (1.69). Whereas, minimum fresh weight of roots (0.81) was recorded under control.

**Dry matter of roots (%)**

The data presented in table 7 shows that the dry matter of roots ranged from 36.30% to 54.50%. The maximum length of roots was observed under *Trichoderma viride* @ 5% of rooting media (54.50%). Whereas, minimum dry matter roots (36.30%) was recorded under control result is in hormony with Sanabria *et al.*, (2014) (Fig. 7b).

**Table.1 Effect of bio control agents on days taken to start sprouting, days taken to 50 % sprouting, success percent**

| Treatment      | days taken to start sprouting | days taken to 50 % sprouting | Success percent |
|----------------|-------------------------------|-------------------------------|-----------------|
| Control        | 12.00                         | 30.67                         | 66.67           |
| *Trichoderma*  | 7.67                          | 26.00                         | 83.33           |
| *Pseudomonas*  | 8.00                          | 27.67                         | 80.00           |

**Table.2 Effect of bio control agents on number of shoots per cutting**

| Treatment      | 30DAP | 60DAP | 90DAP | 120DAP | 150DAP | 180DAP |
|----------------|-------|-------|-------|--------|--------|--------|
| Control        | 3.27  | 4.20  | 4.67  | 5.47   | 6.00   | 6.80   |
| *Trichoderma*  | 5.73  | 6.13  | 6.73  | 7.60   | 8.40   | 9.07   |
| *Pseudomonas*  | 3.60  | 4.27  | 5.27  | 6.20   | 6.60   | 8.20   |

**Table.3 Effect of bio control agent on length of shoots**

| Treatment      | 30DAP | 60DAP | 90DAP | 120DAP | 150DAP | 180DAP |
|----------------|-------|-------|-------|--------|--------|--------|
| Control        | 8.41  | 15.09 | 20.47 | 23.25  | 25.81  | 27.79  |
| *Trichoderma*  | 12.07 | 21.77 | 25.80 | 29.40  | 30.75  | 34.79  |
| *Pseudomonas*  | 11.13 | 19.94 | 23.45 | 28.61  | 30.29  | 33.08  |

**Table.4 Effect of bio control agent on number of leaves per shoots**

| Treatment      | 30DAP | 60DAP | 90DAP | 120DAP | 150DAP | 180DAP |
|----------------|-------|-------|-------|--------|--------|--------|
| Control        | 8.73  | 14.33 | 20.27 | 24.93  | 27.40  | 29.87  |
| *Trichoderma*  | 12.53 | 19.00 | 25.00 | 28.87  | 31.13  | 33.67  |
| *Pseudomonas*  | 11.40 | 17.87 | 24.07 | 28.07  | 30.00  | 32.73  |
Table 5 Effect of bio control agent on total number of leaves per cutting

| Treatment   | 30DAP | 60DAP | 90DAP | 120DAP | 150DAP | 180DAP |
|-------------|-------|-------|-------|--------|--------|--------|
| Control     | 31.40 | 65.33 | 75.33 | 83.60  | 86.67  | 89.40  |
| *Trichoderma* | 40.00 | 78.93 | 89.60 | 94.67  | 98.73  | 100.40 |
| *Pseudomonas* | 38.20 | 75.33 | 86.20 | 92.33  | 95.47  | 97.93  |

Table 6 Effect of bio control agent on survival percentage, Number of roots, Length of roots

| Treatment   | Survival percentage | Number of roots | Length of roots |
|-------------|---------------------|----------------|-----------------|
| Control     | 56.67               | 25.27          | 20.96           |
| *Trichoderma* | 76.67              | 36.47          | 28.35           |
| *Pseudomonas* | 73.33              | 33.4           | 26.19           |

Table 7 Effect of bio control agent on Diameter of roots, Fresh weight of roots, Dry matter of roots

| Treatment   | Diameter of roots | fresh weight of roots | Dry matter % of roots |
|-------------|------------------|-----------------------|-----------------------|
| Control     | 0.73             | 0.81                  | 36.3                  |
| *Trichoderma* | 1.73            | 1.69                  | 54.5                  |
| *Pseudomonas* | 1.53            | 1.45                  | 52.37                 |

Fig 1 Effect of bio control agents on days taken to start sprouting, days taken to 50% sprouting, success percent
Fig. 2 Effect of bio control agents on number of shoots per cutting

Fig. 3 Effect of bio control agent on length of shoots

Fig. 4 Effect of bio control agent on number of leaves per shoots
**Fig. 5** Effect of bio control agent on total number of leaves per cutting

**Fig. 6** Effect of bio control agent on survival percentage, Number of roots, Length of roots

**Fig. 7a** Effect of bio control agent on Diameter of roots, Fresh weight of roots,
It was observed that use of various dose of bio control agents resulted in better root and shoot development of pomegranate hardwood stem cutting in consideration with control. Among the bio control agents used first sprouting of cutting, sprouting of 50% of cuttings, highest success percent, root length, leaf count per longest shoot, fresh root weight, root diameter, dry matter content of root and root count per cutting was found to be maximum with application of *Trichoderma viride* followed by *Pseudomonas fluorescence*.

**References**

Arshad, M. William, T., Frankenberger, J., 1997 Plant Growth-Regulating Substances in the Rhizosphere: Microbial Production and Functions. Advance in Agronomy. 6;45-151

Jaganath, S., Meenakshi, H. C., Harinikumar K. M., Nachegowda, V. 2009. Effect of microbial inoculants on rooting of pomegranate. In Proceedings of II International Symposium on pomegranate and minor including Mediterranean Fruits. University of Agricultural Science. Dharwad. India. Abstract no 72.

Manka, M., Fruzynska, B. A. and Dahm, H.

1997. Promoting effect of *Trichoderma* on cutting growth in biocontrol of *Fusarium* carnation wilt. Folia Horticulturae. ISSN; 0867-1761

Marina, T., Ruocco, M., de Masi, L., de Palma, M., Lorito, M., 2011. The beneficial effect of *Trichoderma* spp. On tomato is modulated by the plant genotype. Molecular Plant Pathology 12(4): 341-354

Negi, J., Manhas, R.K., Chauhan, P.S., 2003 Carbon allocation in different components of some tree species of India: A new approach for carbon estimation. Current Science., 85(11): 1528-1531

Patil, P.B., Patil, C.P., Kumar, S., 2001. Impact of inoculation of microorganisms on rotability of pomegranate cuttings. Karnataka Jornals of Agriculture Science. 14; 1020-1024

Sanabria, A. O. H., Alvarez-Herrera, J.G. 2014. Effect of IBA and *Trichoderma harzianum* Rifai on asexual cape gooseberry propagation (*Physalis peruviana* L.). Agronomia Columbia, 32(3): 326-333.

Seeram, N.P., Adams, L.S., Henning, S.M., Niu, Y., Zhang, Y., Nair, M.G., Heber, D., 2005. *In vitro* antiproliferative,

![Fig. 7b Effect of bio control agent on Dry matter of roots](image_url)
apoptotic and antioxidant activities of 

punicalagin, ellagic acid and a total 
pomegranate tannin extract are 
enhanced in combination with other 
polyphenols as found in pomegranate 
juice. J. Nutr. Biochem. 16(6): 360-367.

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