The Effect of Trichoderma asperellum Tc-Clkt-01 and Shallot Extract on the Success of Grafting in Mango Seedlings

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Abstract. This study aims to determine the effect of Trichoderma asperellum Tc-Clkt-01 and shallot extract and their interaction on the success of grafting mango seedlings. The factorial experiments in this study were arranged in a completely randomized design (CRD). The first factor is Trichoderma consisting of without and with Trichoderma. The second factor was the concentration of shallot extract above: 0, 15, and 30 ml.L\(^{-1}\). With three replications, 18 experimental units were obtained. The variables observed were: shoot appearance time (days) and shoot growth length (mm) one to four weeks after grafting. All data from the observations were analyzed using ANOVA at the 5% level; the average of each treatment was compared with controls (without Trichoderma and without red onion extract). Trichoderma sp. isolate Tc-Clkt-01 accelerated the emergence time of shoots by 26.87% and increased shoot length growth by 41.76-43.71% compared to the control. Onion extract at a concentration of 15 ml.L\(^{-1}\) accelerated the emergence of shoots by 10.45% and increased shoot length increase by 20.59% compared to the control. The combination of Trichoderma and shallot extract at a concentration of 30 ml.L\(^{-1}\) accelerated the emergence of shoots by 23.17% and increased shoot length by 32.94% compared to the control.

1. Introduction

Mango is a horticultural commodity that has good prospects for both national consumption and export. For the sustainability and development of mango cultivation, rejuvenation of plants by providing good quality seeds must always be cultivated.

Vegetative propagation of plants using the grafting method, among others, is designed to combine varieties that are tolerant and resistant to environmental stresses as rootstocks with varieties that have high productivity and an attractive fruit taste as a scion.

To speed up the time of seed production through grafting, especially the emergence of roots and shoots, natural growth regulators are often used as well as synthetic chemical compounds. Onion extract can act as a growth-regulating compound such as gibberellin and auxin which can influence the development of root elongation in the cell elongation process in plants [1] and has the prospect of being applied to various plants considering that these compounds are safe and easily available. However, to what extent the active compounds in onion extract can affect the success of grafting, careful testing is necessary.
On the other hand, wounded plant shoot joints have the potential to be contaminated by microbes, especially plant rot fungi and can cause damage to the injured tissue. To prevent infection and decomposition of contaminant fungi, it is necessary to protect with materials that can inhibit the activity of tissue-destroying microbes. One of the materials used is the biological agent *Trichoderma*.

*Trichoderma* sp. is one type of saprophytic soil fungus that has the ability to suppress through the activity of extracellular compounds it produces and is mycoparasitic by directly attacking and inhibiting pathogenic fungi that cause plant disease [2-4] so that this fungus provides great benefits for plants and able to produce growth-stimulating compounds for plants [5-7].

The similarity of some characteristics between Trichoderma activity and shallot extract is thought to provide synergy in helping the success of grafting. On the other hand, onion extract contains various compounds that can affect the activity of other organisms including the Trichoderma fungus. For this reason, it is necessary to test each of the effects of the onion extract and the Trichoderma fungus on the success of grafting and to test the effect of the extract on Trichoderma activity and the possibility of the occurrence of synergy between the two in supporting the grafting process.

This study aims to determine the effect of *Trichoderma asperellum* Tc-Clkt-01 and shallot extract and their interaction on the success of grafting mango seedlings.

2. Methods

**Preparation and implementation of grafting.** Trichoderma for this experiment was *T. asperellum* isolate Tc-Clkt-01 from the collection of the Laboratory of Microbiology and Biotechnology, University of Muhammadiyah Sidoarjo. The biological agents were reproduced on PDA-m media. After being incubated for 12 days, the culture was harvested and crushed using a blender, and sterile distilled water was added for three minutes to produce a suspension containing Trichoderma propagules. Before use, the spore content was calculated [8] with a population density of $10^5$ CFUml$^{-1}$ Trichoderma and accommodated in 500 ml Erlenmeyer.

The preparation of shallot extract begins with extracting 250 grams of shallot bulbs to produce 200 ml of crude red onion extract. Then take as much as 15 ml and 30 ml and dissolve it with 1000 ml of distilled water and then in the solution until well blended and prepared as a treatment.

To ensure the content contained in the onion extract, a series of extraction is carried out, so that chemical isolates will be obtained which are thought to contain precursor compounds that are useful for the growth of grafting seeds. First, dry the shallots in a cabinet with a temperature of 70ºC for 1 week. Then the shallot is crushed until smooth and weighed until it reaches a weight of 20 grams and 50 ml of hexane is added then boiled for 15 minutes, then filtered. The purpose of giving the hexane solution is to separate the fat from the extract. The results of the onion extract are added with 70% methanol as much as 50 ml shaken for 1 hour. The results are filtered to separate the solution containing allelochemical compounds from the waste. The solution containing allelochemical compounds was added with methanol 70% as much as 50 ml, then evaporated for 30 minutes at a temperature of 45ºC. The chemical isolates that have been obtained are then analyzed using the application of the Gas Chromatography-Mass Spectrophotometry method (QP2010 plus Shimadzu, Japan) [9] so that the composition of the chemical compounds contained in shallot extraction with methanol as a solvent is obtained.

Mango seedlings in polybags measuring 15 x 20 cm prepared as rootstocks are a local variety of mango Gadung which is around 3-4 months old which has good growth, is sprouting, and is healthy. For the scion is the Indian Mango Golek variety which grows well and is not infected by pests and diseases. The rootstock is taken from the mother tree, which is neither too young nor too old, using garden shears. Mango seeds (rootstock) are watered the last time the day before grafting.

The grafting was carried out in the afternoon at 04.00-06.00 pm. The end of the rootstock is cut in the middle towards the base along ± 3cm; meanwhile, the scion is prepared by cutting branches from the Indian mango Golek plant along 10-15 cm or by leaving two to three buds. Next, make an incision like the letter "V" at the base of the upper stem. Both the scion and rootstock were soaked for 30 seconds using shallot extract and Trichoderma suspension according to the treatment using several containers. For the combination treatment, Trichoderma suspension and shallot extract were mixed and stirred evenly for 10 seconds before soaking. After soaking the scion, the scion is then drained for...
15 minutes so that when the grafting is done, the scion is not wet. The upper stem is then inserted into the cleavage on the rootstock and then bonded using a 0.2 mm thick plastic sheet, from bottom to top; the remainder of the ties is used for the hood. Observation of the connection results will be carried out every week between 35-56 days after connection (HSP); Meanwhile, determining the timing of the initial appearance of grafting seedlings was carried out by observing the growth of the seeds every day starting from the seventh day until all the grafting seeds had produced new shoots. Spraying according to the treatment carried out after grafting is carried out by spraying onion extract and Trichoderma suspension every two days in the afternoon aimed at all parts of the plant. To maintain moisture grafting seeds, watering the soil of the planting medium in the polybags is done in the morning (06.00-06.30 am) or evening (05.00-05.30 pm).

**Experimental design and data analysis.** The experiments in this study were arranged factorial in a completely randomized design (CRD). The first factor of the Trichoderma application consisted of without Trichoderma (T0) and Trichoderma isolate Tc-Clkt (T1). The second factor is the concentration of shallot extract, consisting of: 0, 15, and 30 ml L⁻¹, respectively symbolized as E0, E1, and E2. With three replications, 18 experimental units were obtained.

All data from the observations that have been obtained were analyzed using analysis of variance (ANOVA) at the 5% and 1% real levels; Each treatment average value for all observed variables was compared with the difference against the control (without being given Trichoderma and/or shallot extract).

For experimental studies, the observed variables were: (i) the time of shoot emergence (days), and (ii) shoot growth length (mm) on days 7, 14, 21, and 28 days after grafting (DAG)

3. **Results and Discussion**

The results of the Gas Chromatography Mass Spectrophotometry (GCMS) analysis showed the composition of the chemical compounds contained in the onion extract with methanol as a solvent as shown in Table 1.

| No. | Name of compound | Peak area (%) | CAS number* |
|-----|------------------|---------------|-------------|
| 1   | Cyclobutanol     | 1.12          | 002919-23-5 |
| 2   | Ethanone, 1-(1H-pyrrol-2-yl)- | 4.12       | 001072-83-9 |
| 3   | N1,N1-Dimethyl-N2-isobutylformamidine | 0.89       | 123528-99-4 |
| 4   | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- | 11.70     | 028564-83-2 |
| 5   | Cyclopropyl carbinol | 2.93          | 002516-33-8 |
| 6   | Cyclopentanol    | 23.85         | 000096-41-3 |
| 7   | Piperazine       | 14.08         | 000110-85-0 |
| 8   | (tetrahydroxycyclopentadienone)-tricarbonyliron(0) | 1.90       | 117696-75-0 |
| 9   | Benzeneethanamine, N-methyl- | 3.20          | 000589-08-2 |
| 10  | 2,4(1H,3H)-Pyrimidinedione, 5-nitro- | 3.36       | 000611-08-5 |
| 11  | Phenethylamine, N-hexyl- | 1.78          | 024997-83-9 |
| 12  | n-Pentanal       | 3.87          | 000110-62-3 |
| 13  | S-Nitrouracil    | 6.07          | 000611-08-5 |
| 14  | N-Acetyl-L-alanine | 6.71          | 000097-69-8 |
| 15  | Benzeneethanamine, N-methyl- | 10.94       | 000589-08-2 |

* Code number according to CAS (Chemistry Abstract Service)

The analysis of variance showed that the interaction between Trichoderma and shallot extract and the effect of each factor had no significant effect (p>0.05) on the initial time of shoot appearance and
the increase in plant length. The mean effect of Trichoderma and shallot extract application on early appearance time and shoot length of grafting mango seedlings and the percentage increase compared to control (T0E0) are presented in Figures 1 and 2, respectively.

![Figure 1](image1.png)

**Figure 1.** The average effect of *Trichoderma* and shallot extract application on shoots early emergence time. The number (percentage) above the bar shows the percentage increase (+) or decrease (-) in the average increase in shoot length per week compared to the control (x).

![Figure 2](image2.png)

**Figure 2.** The average effect of *Trichoderma* and shallot extract application on shoot length gain at 5-8 weeks after grafting.

The results of GCMS analysis (Table 1) show that the onion extract contains various compounds that can be precursors for the formation of several growth-regulating compounds. Extraction results at various concentrations of methanol solvent show the fractionation of methanol and flavonols [10] which can encourage peroxidase activity which has the potential to promote plant growth [11].

The absence of the interaction effect between shallot extract and Trichoderma is made possible by the presence of antimicrobial compounds contained in shallot extract [12] which can inhibit bacteria and fungi, including the effect on Trichoderma. In addition, in a long period of time in the grafting process of this biological agent fungus, it is suspected that it lacked oxygen considering that in the grafting stage, the joints were coated with plastic sheets. One of the important performances shown by
Trichoderma is supplying plant growth regulators [13-14], which is thought to be not optimal for a long time or more than two weeks of incubation. A grafting process in which the availability of oxygen is depleted under a plastic layer. Meanwhile, it appears that the time of emergence of shoots in the Trichoderma treatment is combined with either no extract or 30 ml of onion extract. L-1 (mean 26.9 and 23.2 days) was faster than control and other treatment combinations. In both treatments, it appears that Trichoderma promotes the acceleration of shoot emergence given its ability to produce secondary metabolites such as peptaibols and harzianolides [15-16] and various other active metabolites that promote the emergence and shoot growth [17-18].

4 Conclusion

Trichoderma sp isolate Tc-Clkt-01 accelerated the emergence time of shoots by 26.87% and increased shoot length growth by 41.76-43.71% compared to the control. Onion extract at a concentration of 15 ml L-1 accelerated the emergence of shoots by 10.45% and increased shoot length increase by 20.59% compared to the control. The combination of Trichoderma and shallot extract at a concentration of 30 ml L-1 accelerated the emergence of shoots by 23.17% and increased shoot length by 32.94% compared to the control.

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