IMPROVED TOLERANCE OF CHILI AGAINST DROUGHT STRESS BY THE USE OF *TRICHODERMA*-BASED COMPOST AND LIQUID FERTILIZER FROM LOCAL MICROORGANISM (LMo)

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Abstract. The present study was aimed to evaluate the effects of six different technology packages to improve tolerance of three chili cultivars for drought stress conditions. Six technology packages namely trichocompost and Local Microorganism (LMo) spray every 7 days, trichocompost and LMo spray every 14 days, trichocompost only, LMo spray every 7 days only, LMo spray every 14 days only, and a control (no trichocompost nor LMo spray) were applied on three chili cultivars (Lado, Laris and Vitra). The trial was arranged in a randomized block design with three replicates consisting of four individual plants. Results indicated that the enrichment of growing media with trichocompost along with spraying of LMo improved the growth and yield of Lado, Laris and Vitra cultivars in drought conditions. Trichocompost in combination with LMo spray at 7-day interval increased the number of productive branches, as well as fruit number and fruit weight up to 28%, 58.84% and 64.28% in the cultivar Vitra, which was higher than the Laris and Lado. The application of *Trichoderma*-based compost plus spraying LMo every 7 days was able to increase tolerant properties of the three chili cultivars for drought stress. Within cultivars, Vitra was proven to be more adaptive and more tolerant against drought stress in comparison with Laris and Lado cultivars.

Keywords: *Capsicum annuum*; biofertilizer; abiotic stress; *Pomacea canaliculata*.

INTRODUCTION

Climate change occurring around the world has caused drought threat in many areas, and plants will suffer from a water scarcity. Drought is an important threat to sustainable agriculture because most of plant physiological processes are water-dependent [36]. Water shortage due to drought will inhibits nutrient uptake causing nutrient deficiency and disrupting cellular metabolism. This will lead to poor plant growth and development, resulting serious loss of yield [1, 26].

In tropical region such as Indonesia, limited soil water availability normally took place during dry season. Therefore, chili (*Capsicum annuum* L.) as many other vegetable crops, grown during dry season will experience drought stress unless water is adequately supplied by any method of irrigation. Ichwan et al. [14] reported that chili grown on soil with 50% field capacity showed slow growth and reduced yield. Also, number of productive branches, number of fruits and total fruit weight per plant were also low.

Drought is a condition of prolonged restricted ground water availability as a result of unfavorable conditions of growing medium. Many metabolism processes of plant were affected by drought stress, either directly or indirectly [38]. Noroozlo et al. [26] suggested that a significant change in cellular metabolism was generally occurred resulting in reduced plant growth and production. In addition to affecting plant physiological processes, drought also affects biochemical processes, anatomy and plant morphology [2].

Efforts to improve soil water availability during limited water supply can be done by the use of organic materials such as *Trichoderma*-enriched compost or trichocompost [6, 18, 22]. The use of organic materials may increase plant tolerance to drought due to better soil structure, increase aeration in root zone, reduce mass density, increase cation exchange capacity, and maintain primary nutrients such as N and P [42]. The application of organic materials in soil has also proven to be beneficial to increase soil water holding capacity. Bhadha et al. [7] reported that an increase of 1% soil organic matter significantly increased soil water holding capacity.

The application of benefecial microorganisms such as *Trichoderma* in growing media is another way to enhance organic matter and improve chemical, physical and biological properties of soil. Microorganisms in compost are generally saprophyte bacteria (chemoorganotrophic and chemolithotrophic groups) and fungi. Many reports on the application of *Trichoderma*-enriched compost to promote plant growth and yield have been found in various horticultural crops such as *Lycopersicon esculentum* [24], *Brassica oleracea* [32] and *Ipomoea reptans* [43].

Bacteria from compost are mostly decomposers, also known as saprophytes, belonging to the group of chemoorganotrophic microorganisms. Among them, bacterial genera like *Pseudomonas, Burkholderia, Zymomonas, Xanthomonas*, and nitrogen-fixing aerobic bacteria can be found. Chemolithotrophic microorganisms can also be found in the compost like nitrifying bacteria that convert the ammonium into nitrites and nitrates. The most representative genera of this type are *Nitrosomonas* and *Nitrospira* [16, 20, 34, 35]. In addition, Fungi are also decomposers in nature and they can be found during the first and final phase of the composting process. The most representative genera are *Aspergillus, Acremonium, Chrysosporium, Fusarium, Mortierella, Penicillium,* and *Trichoderma* [3, 29].

Trichoderma was found to have antagonistic potential against root pathogenic isolates, so that it can inhibit or even kill pathogenic fungi and increase plant tolerance to diseases caused by soil-borne pathogens [8, 9, 25, 41]. Therefore, trichocompost can be used as an alternative of organic fertilizers to increase plant growth and production while increasing crop protection.

Applying a liquid of local microorganisms (LMo) through foliar spray could increase the performance of plants grown on *Trichoderma*-enriched media as reported by many authors [10, 32, 43]. The LMo is a liquid containing various microorganisms involved in the overhauling of various organic wastes [27]. The main ingredients of LMo are carbohydrates, glucose, and a source of beneficial microorganisms, so they are recommended as decomposers, organic fertilizers, or biological pesticides. The ability of microorganisms in LMo to decomposition has been widely used for activation in the composting process [5, 21, 27].

One of potential source of ingredients of LMo is golden apple snail (*Pomacea canaliculata* Lamarck). It is a freshwater mollusk which is an important pest for rice cultivation in Indonesia. However, this snail is also able to provide benefits for farmers by processing it into LMo ingredients. LMo which is made from golden apple snails contains growth hormones that stimulate plant growth. In addition, LMo made of golden apple snails also contains nitrogen-fixing bacteria such as *Azospirillum, Azotobacter* and *Pseudomonas,* phosphate solubilizing microbes as well as microbes that play a role in the process of breaking down macromolecules into basic materials, various enzymes and growth hormones that are useful for plants [4, 17, 30].

The use of *Trichoderma* in the decomposition of organic matter may accelerate the composting process, as well as improve plant root system, and thereby increasing the ability of plants to extract water from soil. When combined with the application of LMo, it is believed that the technology will be able to overcome the lack of nutrient availability while increasing plant tolerance to drought stress. To our knowledge, there has no simultaneous application of trichocompost and LMo golden apple snail as a technology package in organic cultivation of chili was found, especially in the chili production centers in Jambi Province, Indonesia. Therefore, the use of this technology package could be a strategic step to support organic chili production in Jambi Province, and is worthy of further investigation.

MATERIAL AND METHODS

Experimental design and treatments

The study was conducted at the Teaching and Research Farm Faculty of Agriculture University of Jambi, Indonesia, from April 2020 through to September 2020. A factorial randomized block design with three replicates was employed in the trial. The variables tested were six technology packages (trichocompost and LMo spray every 7 days, trichocompost and LMo spray every 14 days, trichocompost only, LMo spray every 7 days only, LMo spray every 14 days only, and no trichocompost nor LMo spray as control) that were applied on three chili cultivars (Lado, Laris and Vitra). Therefore, there were 18 treatment combinations.

LMo and trichocompost preparation

The LMo is fermented liquid made from natural ingredients containing microorganisms with the potential to transform organic matter, stimulate plant growth, and control pests and plant diseases. In this study, the mixture of LMo was obtained from golden apple snail, a fresh water mollusk, which is an important pest for rice cultivation in Indonesia. Some non-pathogenic microorganisms contained in a LMo solution are *Bacillus* sp., *Sacharomyces* sp., *Azospirillum* sp., *Azotobacter* sp., *Pseudomonas* sp., *Aspergilus* sp. and *Lactobacillus* sp.

The LMo was made by preparing 5 kg crushed snails and 1 kg brown sugar into 10 L coconut water, stirred and mixed thoroughly. The mixed material was kept in a closed container connected to a small bottle filled with water through a small hose to neutralize gaseous materials formed due to fermentation, then left for approximately 2 weeks before use. A well-prepared LMo solution is characterized by the absence of a foul odor, and is ready to be applied to plants.

This study used *Trichoderma harzianum* isolated from soil samples which previously were used to grow chili. The isolation and propagation protocol followed Wagner [39] method. One gram of soil sample from root area was mixed with 9 mL of sterile water in a test tube and stirred evenly. A serial dilutions were carried out so that the population could be calculated. One milliliter of the soil sample suspension was mixed with Potato Dextrose Agar (PDA) medium, and incubated at room temperature. The identification and morphological characterization of *Trichoderma* was determined using determination key of Watanabe [40] until the isolate was identified. The isolates of *T. harzianum* were then purified in PDA medium, and incubated for 7 days at room temperature. The whole series of work was done aseptically.

Trichocompost materials were prepared by mixing vegetable wastes, husk charcoal and cow manure in a definite proportion. Dolomite lime was added to maintain the pH neutral. A solution of brown sugar plus Effective Microorganism-4 (EM-4) were drenched to the materials, mixed thoroughly, and leave fermented for one week. After one week of fermentation, *T. harzianum* isolates were added to the materials, completely mixed, and keep fermented for next three weeks. Following three weeks of fermentation the *Trichoderma* fungus must have grown indicated by the appearance of fine white hyphae on the surface of materials, and this is the trichocompost which is ready to use as biofertilizer.

Plant preparation

Seeds were soaked in LMo solution for approximately 1 hour to induce uniform germination. Seeds were sown on a mixture of soil + trichocompost + sand (2:1:1) in a plastic pot. Spacing up to two seeds per pot was done at 2 weeks after germination, and following 4 weeks of germination the seeds were ready to be transplanted to black polyethylene bags (polybags) in greenhouse.

The growing medium in the greenhouse was 10 kg mixture of soil and trichocompost with a ratio of 1:1. The LMo was sprayed at 500 mL per plant every 7 or 14 days according to treatment. Routine plant maintenance such as pest and disease control, weeding and water control were carried out in order to keep plants in drought conditions.

Plants were subjected to drought from the beginning of transfer to polybags in the greenhouse (0 day after transplanting). The drought condition was made by keeping soil water content at 75% of field capacity, which was obtained through gravimetric measurements [37]. This was done by pouring water until reaching saturation, and water was let to drain for two days. The difference between initial weight of the polybags and final weight after draining indicated the amount of water given to soil to reach field capacity condition. If the amount of water was 100 mL to reach field capacity, so the amount of water to reach 75% of field capacity would be 75 mL. The volume of water was applied to the plant every two days to keep drought condition.

Data collected and statistical analysis

Observations and data collected were made on the following variables: plant height (cm), number of productive branches, days to flowering, number of fruits per plant, weight of fruit per plant (kg), and relative water content. Except for RLWC, data on the effect of trichocompost treatment and LMo spraying were statistically analyzed using Analysis of Variance, followed by Duncan’s Multiple Range Test to see the difference in the effect among treatment means.

Plant height was measured at 12 weeks after transplanting from the root neck to the tip of the stem; number of productive branches was measured when dichotomous began to appear until 12 weeks after transplanting; days to flowering was measured when the first flower appeared on the sample plants; number of fruits per plant was measured by adding up all fruits obtained from the first to the last harvest; weight of fruit per plant was measured by weighing all fruits yielded from the first to the last harvest.

Relative leaf water content (RLWC) was determined according to González and González-Vilar [11] method. Young leaf samples were taken randomly from 3 sample plants of 3 replicates at 10 weeks after transplanting. Six sections of leaves (apical and basal parts were removed) from a same plant were put in a pre-weight tube, sealed and placed in 10^{o}C to prevent growth and evaporation. Tubes with tissue sections inside were weight to give a value for tissue fresh weight (FW). Once every sample has been weighed, a small volume of distilled water was added. Tubes were placed a fridge for 24 hours to allow the tissue taking up water. The tissue sections were taken away from distilled water, the excess of water on the leaf surface were removed carefully with tissue paper, and put in a pre-weight tube to give a measure of fully turgid fresh weight (TFW). The opened-tubes were then placed in an drying oven (70^{o}C) for 24 hours, then the tissues were reweigh to obtain a value for dry weight (DW). Relative leaf water content (RLWC) was then calculated using the following equation:

$$RLWC = \frac{FW - DW}{TFW - DW} \times 100$$

RESULTS

Data on experimental variables were statistically analyzed using analysis of variance (ANOVA) to detect the effect of different cultivars and technology packages on growth and production of chili. The results of the ANOVA is summarized in Table 1.

Plant height

The ANOVA presented in Table 1 indicates that both cultivars and technology packages significantly affect plant height, but the interaction of these two factors does not show any significant effect on plant height. The effect of different cultivars and technology packages on plant height is presented in Table 2 and Table 3.

Table 2 shows that Laris and Vitra significantly grow higher than Lado in drought conditions. This indicates that Laris and Vitra are more adaptable to sub-optimal or drought conditions than Lado. In drought conditions, the presence of *Trichoderma*-based compost in the growing medium and spraying LMo at intervals of 7 or 14 days were able to increase the growth of chili plants by 15% (96.16 cm) and 8% (90.60 cm) respectively, compared to without compost nor local microorganisms (83.68 cm) as indicated in Table 3.

Table 1. Summary of analysis of variance (ANOVA) the effect of technology packages (trichocompost + LMo) and cultivars on growth and production variables of chili grown under drought condition (** = highly significant; * = significant; ns = non-significant)

Source of Variations	Plant height (cm)	Number of productive branch	Days to flowering	Number of fruit	Weight of fruit (g)
Block	**	ns	*	ns	ns
Plant Cultivars (C)	**	ns	ns	**	**
Technology Package (T)	**	**	ns	**	**
Interaction (T × C)	ns	ns	ns	**	**
Coefficient of Variance (%)	5.86	10.07	22.35	6.44	7.29

Productive branches

The ANOVA presented in Table 1 shows that cultivars do not significantly affect the number of productive branches. In contrast, the technology packages show a significant effect on the number of productive branches. However, the interaction between these two variables do not show any significant effect on the number of productive branches. The effect of technology packages on the number of productive branches is presented in Table 4.

Table 4 shows that under drought conditions, growing media enriched with trichocompost is able to increase the number of productive branches by 29% (118.76) to 38% (127.30) compared to control (92.03) either with or without spraying of LMo.

Days to flowering

Days to flowering was recorded at the earliest time of floral development and flower buds can been seen with bare eyes on leaf axil. The ANOVA presented in Table 1 shows that neither cultivars nor technology packages nor interaction of these two variables significantly affect days to flowering.

Number of fruits

The ANOVA presented in Table 1 indicates that either technology packages or cultivars or interaction of these two variables significantly affect chili production in term of number of fruit. The effect of different cultivars and technology packages as well as the interaction of the two variables on the number of fruit production is presented in Table 5.

Table 5 shows that enriching growing medium with trichocompost and spraying LMo could increase the average number of fruits per plant under drought conditions. In general, Vitra shows better response than Lado and Laris cultivars. The highest average number of fruit per plant is shown by Vitra grown on medium enriched with trichocompost and sprayed with LMo at 7-day interval (87.67), followed by 14-day interval (82.00).

Table 2. The effect of different cultivars on the height of chili plants grown under 75% field capacity

Chili cultivars	Average plant height (cm)
Laris	96.68 a
Vitra	96.31 a
Lado	77.11 b

Numbers followed by the same lowercase indicate a non-significant different based on Duncan's Multiple Range Test at α = 0.05.

Table 3. The effect of different technology packages on the height of chili plants grown under 75% field capacity

Technology packages	Average plant height (cm)
Trichocompost and LMo spray at 7-day interval	96.16 a
Trichocompost and LMo spray at 14-day interval	90.60 b
LMo spray at 7-day interval	90.37 b
Trichocompost only	89.87 b
LMo spray at 14-day interval	89.52 b
Control	83.68 c

Numbers followed by the same lowercase indicate a non-significant different based on Duncan's Multiple Range Test at α = 0.05.

Table 4. The effect of different technology packages on the number of productive branches in three chili cultivars grown under 75% field capacity

Technology packages	Average productive branch
Trichocompost and LMo spray at 14-day interval	127.30 a
Trichocompost only	120.63 a
Trichocompost and LMo spray at 7-day interval	118.76 a
LMo spray at 7-day interval	101.99 b
LMo spray at 14-day interval	99.36 b
Control	92.03 b

Numbers followed by the lowercase indicate a non-significant different based on Duncan's Multiple Range Test at α = 0.05.

Table 5. The effect of different technology packages and different cultivars on fruit number of chili grown under 75% field capacity

Technology packages	Chili cultivars		
	Lado	Laris	Vitra
Control	26.00 c F	32.33 b F	35.67 a E
LMo spray at 7-day interval	45.33 b E	42.67 c E	50.00 a D
LMo spray at 14-day interval	52.00 a D	47.00 c D	48.00 b E
Trichocompost only	67.67 b C	60.67 c C	74.00 a C
Trichocompost and LMo spray at 7-day interval	79.33 b A	67.67 c A	87.67 a A
Trichocompost and LMo spray at 14-day interval	73.67 b B	63.33 c B	82.00 a B

Numbers followed by the same lowercase in rows and the same uppercase in columns indicate non-significant different based on Duncan's Multiple Range Test at α = 0.05.

Table 6. The effect of different technology packages and different cultivars on fruit weight (g) of chili grown under 75% field capacity

Technology packages	Chili Varieties		
	Lado	Laris	Vitra
Control	79.67 b F	99.17 a F	114.03 a E
LMo spray at 7-day interval	145.53 b E	142.27 b E	170.83 a C
LMo spray at 14-day interval	170.30 a D	152.25 b D	150.65 b D
Trichocompost only	233.17 b C	191.67 c C	267.25 a C
Trichocompost and LMo spray at 7-day interval	303.43 b A	248.55 c A	318.26 a A
Trichocompost and LMo spray at 14-day interval	295.13 b B	231.32 c B	305.68 a B

Numbers followed by the same lowercase in rows and the same uppercase in columns indicate non-significant different based on Duncan's Multiple Range Test at $\alpha = 0.05$.

Table 7. The effect of different technology packages and different cultivars on relative leaf water content (%) of chili grown under 75% field capacity

Technology packages	Chili Varieties		
	Lado	Laris	Vitra
Trichocompost and LMo spray at 14-day interval	71.81 ± 1.905	79.17 ± 1.801	86.44 ± 1.183
Trichocompost and LMo spray at 7-day interval	67.91 ± 1.238	72.02 ± 1.799	84.23 ± 2.170
Trichocompost only	62.97 ± 1.158	63.32 ± 0.400	77.20 ± 1.004
LMo spray at 7-day interval	61.57 ± 1.697	57.44 ± 1.706	78.69 ± 1.198
LMo spray at 14-day interval	61.23 ± 1.132	55.04 ± 3.290	76.00 ± 2.797
Control	57.72 ± 1.401	54.25 ± 3.614	61.70 ± 1.053

Relative leaf water contents was determined on individual leaves taken from 3 sample plants of 3 replicates at 10 weeks after transplanting.
± Standard Error

Weight of fruits

The ANOVA presented in Table 1 indicates that either technology packages or cultivars or interaction of these two variables significantly affect chili fruit weight. The effect of different technology packages and cultivars as well as the interaction of the two variables on the average fruit weight is presented in Table 6.

Similar to the average number of fruits per plant, enrichment of growing medium with *Trichoderma*-based compost and spraying LMo also increased average fruit weight per plant as presented in Table 6. The greatest fruit weight (318.26 g) was obtained by Vitra cultivar which was planted on medium enriched with *Trichoderma*-based compost and sprayed with LMo every 7-day, followed by spraying every 14-day intervals (305.68 g) on the same medium.

Relative leaf water content

Data presented in Table 7 indicate that under limited soil water availability there is variation in relative leaf water content (RLWC) among different technology packages and chili cultivars. The cultivar Vitra has a higher RLWC (77.38%) than Lado (63.87%) and Laris (63.54%). A higher RLWC indicates a quick plant physiological response against extreme environmental conditions such as drought. All chili cultivars show a higher RLWC when they are grown on media enriched with *Trichoderma*-based compost and sprayed with LMo at either 7- or 14-day intervals (79.14% and 74.72%, respectively). This indicates that the availability of *Trichoderma*-based compost and the application of LMo could increase physiological response of plants grown under drought condition.

DISCUSSION

Many reports indicated that soil water availability during drought could be improved by the use of *Trichoderma*-enriched compost or trichocompost [6, 18, 22]. In our study, the application of trichocompost along with LMo technology packages had a significant effect on plant height, number of productive branches, number of fruits and fruit weight of the three chili cultivars grown under drought conditions. There was also a significant effect of the interaction between the cultivars and LMo technology packages on fruit number and fruit weight (Table 1). However, neither cultivars nor technology packages nor their interactions showed significant effect on flowering. Days to flowering of the three chili cultivars ranged from 26 to 28 days after planting. In normal conditions and based on the descriptions of the cultivars, the average days to flowering of Lado, Laris and Vitra were 25 to 30 days after planting. This indicates that drought did not affect floral growth and development of the three chili cultivars when trichocompost and LMo were applied. However, Mitra [23] stated that days to flowering was dependent on altitude and genetic characteristics. This is in accordance with reports of Lembang Vegetable Research Institute [19] that days to flowering of chili cultivars grown in lowlands was approximately 28 days after transplanting and in uplands ranging from 34 to 39 days after transplanting.

This study revealed that the cultivar Vitra was more tolerant to drought stress than Laris and Lado. This was indicated by higher fruit number and fruit weight which were nearly as much as normal growing conditions. On the other hand, the Lado produced lower fruit number and fruit weight than Laris and Vitra (Tables 5 and 6). This means that Lado is a less tolerant cultivar against drought stress, while Laris is moderately tolerant.

The application of trichocompost along with LMo spray at 7-day interval was able to optimize the development of productive branches and fruit formation in the three chili cultivars grown under drought conditions. This is in accordance with the finding of Hasyim et al. [12] and Rizki et al. [31] who reported that trichocompost could increase the weight of fruit per plant by more than 70% in chili.

Genotype is an important factor in building plant tolerance to drought stress. In line with this, Rosmaina et al. [33] studied the indicators of drought stress tolerance based on correlation and cross-analysis of normal condition (100% field capacity) and stress conditions (75%, 50% and 25% field capacity). It was found that the number of fruit had a positive correlation and had a direct effect on fruit weight per plant, so that it became a phenotypic character that could be used as a selection criterion for drought tolerant chilies. Thus, cultivars that are able to grow and produce higher fruit number and fruit weight under drought conditions can be categorized as drought stress tolerant cultivars.

The application of trichocompost-enriched media along with LMo spray at 7-day interval was able to increase the number of fruit by up to 58.84% and fruit weight by up to 64.28% in Vitra compared to control. This yield was higher than those achieved in Lado and Laris cultivars. This also indicates that the use of trichocompost coupled with LMo spray every 7 days could reduce the adverse effects of drought stress in the three cultivars, especially in Vitra. This is in accordance with the findings in previous study that foliar spray of water soluble nutrients and biostimulants was proven to increase the ability of the leaves to maintain turgor pressure allowing the nutrients contained in the solution to enter the stomata [13, 14, 15].

In drought stress conditions, Vitra was found to be more tolerant than Laris or Lado. This indicated by higher number of productive branches, number of fruit, and fruit weight. This tolerance response was also reinforced by a high relative leaf water content (RLWC) (nearly 80%) or close to stress-free conditions, indicating that Vitra had better drought stress tolerance than the other two genotypes. This was in line with Okunlola et al. [28] report that drought stress conditions significantly reduced RLWC values in *C. chinense, C. annum* and *C. frutesense*. Plants grown under normal condition had an RLWC of 93.75% - 95.05%, plants under light drought stress had an RLWC of 90.60% - 93.40%, plants under moderate drought stress had an RLWC of 69.30% - 80.90%, and plants under severe drought stress had an RLWC of 53.25% - 68.95%.

In conclusion, our study showed that the enrichment of growing media with *Trichoderma*-based compost (trichocompost) along with spraying local microorganisms (LMo) were important to improve tolerant properties of three chili cultivars (Lado, Laris and Vitra) for drought stress. Under drought stress condition, trichocompost and routine spray of LMo increased the number of productive branches, number of fruits and weight of fruits by 28%, 58.84% and 64.28% respectively in Vitra, which was higher than Laris and Lado. This indicates that Vitra is a more adaptive and more tolerant cultivar than Laris and Lado cultivars for drought stress.

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