

Bacterial Inoculants to Increase the Biomass and Nutrient Uptake of *Tithonia* Cultivated as Hedgerow Plants in Ultisols

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ABSTRACT

Ultisols require greater amounts of fertilizer application compared to other soils. Unfortunately, the price of synthetic fertilizers has increased over time during the years, making them unaffordable for most Indonesian farmers. Over the last century, efforts to reduce reliance on synthetic agro-chemicals have recently focused on *Tithonia diversifolia* as a green manure alternative. Generally known by its common name of *tree marigold* or *Mexican sunflower*, this plant has attracted considerable attention for its prolific production of green biomass, rich in nitrogen, phosphorous and potassium (NPK). This outstanding feature and the plant's capacity to solubilize soil P have recently been capitalized for improving the fertility of highly leached soils in Africa and particularly in Kenya. As microorganisms are expected to play an important role in biomass production and high nutrient uptake of this plant, this issue of importance was pursued further in the following investigation. The aim of this study was to determine the type of bacteria suitable for *Tithonia* cultivation as hedgerow plants in Ultisols which have higher biomass production and nutrient content. The field experiment was conducted with 5 treatments in a randomized block design (RBD) using 3 replications. The treatments were: without microorganisms inoculation or control (K); phosphate solubilizing bacteria (PSB) (L); *Azospirillum* (M); PSB + *Azospirillum* (N); and PSB + *Azospirillum* + *Azotobacter* (O). The bacterial substrates were inoculated into the *Tithonia* rhizosphere in the nursery. The young *Tithonia* plants were then planted as hedgerow on Ultisols in the experimental field for 8 months, and pruned once every 2 months. The differences between treatments were statistically significant by HSD test at the 95% level of probability. Treatment L (phosphate solubilizing bacteria) was found to be the most effective, followed by treatment N (PSB + *Azospirillum*).

Key words: *Azospirillum*, *azotobacter*, green manure, PSB, *Tithonia* hedgerow plants

INTRODUCTION

Ultisols occupy approximately 45.8 million ha of the total land area in Indonesia (Subagyo *et al.* 2004; Tan 2008). Based on distribution and size, these soils are perhaps the next highest in land area compared to Inceptisols (the most important

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soils in Indonesia), a clear indication of the great potential of Ultisols for expanding agricultural operations in the country (Tan 2008). However, as Ultisols are acidic and highly leached, they exhibit several constraints such as Al-toxicity and low nutrient content for plant growth. These soils, therefore, require large amounts of fertilizer applications for crops to produce high yields. As prices of synthetic fertilizers have risen considerably, they are becoming unfortunately unaffordable for the common farmers in Indonesia. Therefore, an alternative fertilizer, which is less expensive and easier to produce, is urgently needed to replace synthetic fertilizers without harming the growth and yield of crops grown in Ultisols.

Previous research involving *Tithonia diversifolia*, a plant generally known by its common name of *tree marigold* or *Mexican sunflower* in its native country, Mexico, and locally known in Indonesia as *kembang bulan* or *kembang matahari*, shows great potential for cultivation and uses as green manure for crop production (Figure 1).



Figure 1: Blooming *Tithonia* (left) and as hedgerow plants (right)

The plant, belonging to the aster family (*Asteracea*), exhibits a capacity for producing huge amounts of green biomass, rich in NPK; it is also said to be capable of solubilizing soluble P compounds in soil. The NPK content of tithonia foliage is reported to be greater than cow or stable manure. According to Jama *et al.* (2000), *Tithonia* contains approximately 3.5 to 4.0 % N, 0.35 to 0.38% P, 3.5 to 4.1% K, 0.59% Ca and 0.27% Mg. *Tithonia* plants have recently been used extensively by African farmers in Kenya for improving the fertility level of their highly leached soils (Nziguheba *et al.* 2002; Waniku and Kimenyi 2006; Sanchez and Jama 2000) have also cited reports of *Tithonia* being used by farmers in Kenya as mulch providing NPK for growing corn on their nutrient deficient soils.

Hakim (2002) noted that *Tithonia* grew abundantly at all elevations, even near highways and streets in West Sumatra, Indonesia. However, it has often been considered as a weed and has not found application as an organic fertilizer. Hakim and Agustian (2003) believe that although *Tithonia* does not belong to the leguminous family, the NPK rich biomass qualifies it to be used as green manure.

Tithonia has been shown to reduce the use of synthetic N and K fertilizers up to 50% for the cultivation of chili, ginger, tomatoes, and melons grown in Ultisols in pot experiments by Hakim *et al.* (2003; 2004). Based on results of field studies conducted over two years, Hakim and Agustian (2004; 2005a; 2005b) concluded that growing Tithonia as hedgerows was more effective as an *in situ* or *in vivo* fertilizer alternative in intercropping systems.

The Tithonia plant can be reproduced easily by vegetative means, using stems cuttings, and planted at a spacing of 50 cm x 50 cm in 100 cm wide hedgerows. The appropriate system pattern of Tithonia cultivation is by growing several hedgerows, separated by a distances of 5 m between rows (20 rows = 2000 m² row ha⁻¹). Pruning is required once every two months. With this cultivation technique, Tithonia is noted to produce 6.6 to 6.8 metric tons of dry matter (DM) or about 40 metric tons of fresh biomass, with nutrient contents of 150-240 kg N and 156-245 kg K ha⁻¹ annually. The amount of nutrients released from Tithonia is sufficient to provide 50% of N and K needed by crops grown within each alley. Hakim and Agustian (2004) reported that the amounts of synthetic N and K fertilizer applications for chili and ginger crops could be reduced by as much as 50% under field trials by intercropping with Tithonia hedges. Results from field studies for corn in Ultisols also suggest that application of N and K fertilizers could be reduced by 50% with Tithonia treatment as green manure. Tithonia biomass used as green manure, containing 100 kg N and 100 kg K per 4 ton of dry matter, appear to be sufficient for growth of corn. Corn yield from plants receiving treatments with Tithonia +50% synthetic fertilizers, was significantly higher than that treated with 100% synthetic fertilizers.

Hakimet *et al.* (2007) reported that the high DM yield and nutrient uptake of Tithonia was due to the activities of microorganism in the rhizosphere. They found three isolates of arbuscular mycorrhizal fungi, three isolates of phosphate solubilizing fungi, three isolates of *Azotobacter*, four isolates of phosphate solubilizing bacteria, and three isolates of the bacterial phytohormone producer. Asman *et al.* (2008) who conducted pot experiments found four from the ten treatments produced equal amounts of DM yields and nutrients, namely: (1) phosphate-solubilizing bacteria (PSB), (2) *Azospirillum*, (3) PSB + *Azospirillum*, and (4) PSB + *Azotobacter* + *Azospirillum*. Based on the results of pot experiments, the four bacterial inoculants treatments were tested in the field on an Ultisol. The aim of our study was to determine suitable bacteria for use in cultivating Tithonia as hedgerow, and consequently produce higher biomass and nutrients for Ultisols.

MATERIALS AND METHODS

A field experiment was conducted on an Ultisol at the experimental farm of the University of Andalas, Limau Manis, Padang, located at 250 m a.s.l and 30 km to the east of Minangkabau International Airport, West Sumatra, Indonesia. Selected soil chemical analyses were as follows: pH (H₂O) 5.15, exchangeable-Al 1.43 cmol kg⁻¹, total-N 0.27%, available-P 15 mg kg⁻¹, and exchangeable-K 0.78 cmol kg⁻¹. The mean annual temperature was approximately 26° C and the annual

precipitation was 5546 mm. The treatments were based on data of Asman *et al.* (2008) with the experimental factors being arranged in a randomized block design (RBD) with 5 treatments and 3 replications. The treatments were inoculation of microbial substrates into *Tithonia* rhizospheres of plants grown in the nursery, namely: without microorganisms inoculation or control (K); phosphate solubilizing bacteria (PSB) (L); *Azospirillum* (M); PSB + *Azospirillum* (N); and PSB + *Azospirillum* + *Azotobacter* (O).

Sterilized soil (1 kg) was transferred into a small plastic pot, and then planted with *Tithonia* cutting stem, each having three buds. One bud was buried into the soil, while the others were above the soil surface. After 2 weeks, when leaves and roots had emerged, 10 ml of microbial inoculants of a specific treatment were injected into the root zone as mentioned earlier. The microbial inoculants were prepared in *Nutrient Broth* containing 10^8 bacteria cells per ml. For acclimatization of microbial inoculants with sterilized media for growing *Tithonia*, they were left incubated in the media for one week. The young plants were then fertilized and watered daily with red Hyponex solution (5g red Hyponex in 1 litre of sterile distilled water). After one month in the nursery, the *Tithonia* seedlings were ready to be transplanted into the field.

The size of each experimental plot of hedgerow was 2 m x 0.8 m (1.6 m²) with a planting distance of 50 cm x 50 cm, with 8 seedlings being planted per plot. To stimulate early growth, each planting hole was fertilized with 0.5 kg cow dung manure, 2.5 g N + 0.25 g P + 2.5 g K + 0.25 g Mg as recommended by Hakim and Agustian (2005b). Plants were maintained and observed for 8 months, and pruned every 2 months, (altogether 4 prunes). The biomass of each prune was weighed, and sampled for moisture content determination and dry weight by oven drying at 60°C. Chemical analysis was performed for N, P, and K contents. The differences between treatments were statistically analyzed by HSD test at the 95% level of probability.

RESULTS AND DISCUSSION

The effects of bacterial inoculation on DM yield of *Tithonia* planted in Ultisols as hedgerow with 4 prunes over a growing period of eight months compared to the estimated yield with six prunes annually are presented in Table 1.

The data showed that treatments with bacterial inoculants increased DM yield of *Tithonia* significantly compared to the control (treatment K). The highest increase was obtained by treatment L (inoculation with PSB), showing a total DM yield of 3.76 kg m⁻², which translates into an increase of 47% with an average of 4 prunes. The frequency or cycle of pruning seems to affect the yield of DM, which shows an increase of 85% at the first pruning (Pruning I), to reach maximum DM yields of 116% compared to the control at the second pruning (Pruning II). The dry matter yield decreases in the third pruning (Pruning III), but again reaches higher values of DM yield at the last pruning (Pruning IV). It appears that DM production and yield, in the presence of PSB, increases with successive pruning.

TABLE 1

Dry matter (DM) of Tithonia shoots as influenced by bacterial inoculation re-inoculation into Tithonia rhizosphere in Ultisols, Padang Indonesia

Treatments	Pruning I	Pruning II	Pruning III	Pruning IV	Total 4 prunes	*If 6 prunes a year
(kg m ⁻²).....					t. ha ⁻¹ y ⁻¹
(K) Without bio-agent	0.40 c	0.62 b	0.71 a	0.83 b	2.56	7.68
(L) Phosphate-solubilizing bacteria (PSB)	0.74 a	1.34 a	0.47 c	1.21 a	3.76	11.28
(M) <i>Azospirillum</i> (Azos)	0.55 b	0.64 b	0.54bc	0.66 c	2.39	7.17
(N) PSB + Azos	0.49 b	0.64 b	0.59 b	0.81 b	2.53	7.59
(O) PSB + Azos+ <i>Azotobacter</i> (Azot)	0.50 b	0.69 b	0.74 a	0.89 b	2.82	8.46

Note: Means in the same column followed by different letters are significantly different according to HSD

At 5% probability level

* If pruned 6 times a year on a 2000m² hedgerow ha⁻¹

Based on the average DM yield of 4 prunes (Table 1), it can be concluded that inoculation of Tithonia rhizosphere with PSB was the best treatment for promoting plant growth as it provided the highest DM yield of 3.76 kg m⁻², an amount equal to 7.52 metric ton ha⁻¹ (20 lines of hedgerow = 2000 m² ha⁻¹). With 6 prunes a year, as recommended by Hakim and Agustian (2005b), the yield was 11.3 metric ton DM ha⁻¹y⁻¹, whereas the inoculation by PSB + *Azospirillum* only produced 7.6 metric ton DM ha⁻¹ y⁻¹.

The yield of 7.6 to 11.3 t DM ha⁻¹ y⁻¹ was high compared to the yield of plants without inoculation (6.8 t ha⁻¹y⁻¹) as reported by Hakim and Agustian (2005b). It showed that inoculation with PSB played an important role in the growth of Tithonia to induce higher amounts of DM. The results were clearly due to improved nutrient uptake by crops. Statistical analysis showed that nutrient uptake by Tithonia as hedgerow for 4 prunes was significantly influenced by bacterial inoculation (Table 2). The increase in P uptake was sufficiently high to stimulate better root growth, which then allowed higher nutrient uptake. Consequently, all the above resulted in improved growth and DM yield.

TABLE 2

Total N, P, and K of Tithonia shoots as influenced by bacterial inoculants re-inoculation into Tithonia rhizosphere in Ultisols, Padang, Indonesia

Treatments	Total yield of N, P, dan K for 4 prunes (g. m ⁻²), and estimation for 6 prunes a year (kg.2000 m ² .ha ⁻¹ .y ⁻¹)*					
	N(g. m ⁻²) (kg.ha ⁻¹)	P(g. m ⁻²) (kg.ha ⁻¹)	K(g. m ⁻²) (kg.ha ⁻¹)			
(K) Without bio-agent	55.0 b	165.0	6.3 b	18.9	30.0 c	90.0
(L) Phosphate-solubilizing bacteria (PSB)	71.9 a	215.7	10.0 a	30.0	84.4 a	253.2
(M) <i>Azospirillum</i> (Azos)	63.1 b	189.3	8.8 a	26.4	40.6 b	121.8
(N) PSB + Azos	75.0 a	225.0	10.0 a	30.0	47.5 b	142.5
(O) PSB + Azos+ <i>Azotobacter</i> (Azot)	72.5 a	217.5	8.8 a	26.4	40.6 b	121.8

Note: Figures in the same column followed by different letters are significantly different according to HSD at

5% probability level

* If pruned 6 times a year on a 2000m² hedgerow ha⁻¹

In this study, the uptake of N, P, and K was observed for 8 months from the time of bacterial inoculation to plants subjected to 4 prunes. Hakim and Agustian (2005b) have shown previously that Tithonia could be pruned up to 6 times a

year, and its yield projected or estimated using data from Tithonia DM production pruned 4 times annually as a base (Table 2). In the case of 6 pruning cycles, the area planted with Tithonia was 2000 m² ha⁻¹ (20 hedgerow ha⁻¹ having 1 m width).

The data in Table 2 shows that Tithonia inoculated with PSB, pruned 6 times a year produced approximately 216 kg N, 30 kg P, and 253 kg K ha⁻¹y⁻¹. On the other hand, inoculation with PSB + *Azospirillum* resulted in higher N nutrient content, amounting to 225 kg N, 30 kg P, and 143 kg K ha⁻¹ y⁻¹.

The results from this study were better than that reported earlier by Hakim and Agustian (2005b), where lower nutrient content was observed with about 150 kg N and 156 kg K ha⁻¹ y⁻¹ in biomass of Tithonia, pruned 6 times a year, but without microbial inoculation to Tithonia rhizospheres grown as hedgerows at 5 m distance. Thus, it is clear from the above that inoculation with PSB or PSB in combination with *Azospirillum* was effective in increasing N, P, and K uptake by Tithonia grown as hedgerows. The increased nutrient uptake improved Tithonia growth, resulting in high DM production, which can be used as an organic fertilizer or an alternative fertilizer, replacing or reducing synthetic fertilizer application.

Higher N, P, and K content of Tithonia due to inoculation of PSB is supported by results of previous studies. Alexander 1977; Rao 1982; Illmer *et al.* 1995 reported that the presence of PSB is due to the production of organic acids such as citric acid, glutamate, succinate, lactate, oxalate, glycosilate, malic, fumaric, tartaric, and ketoglutarate. It is believed that the organic acids are effective in solubilizing P, making it more available to plants. The phosphate solubilizing mechanism by PSB can be explained in several ways: (1) changing the solubility of inorganic P compounds, (2) enhancing organic and inorganic P release, and (3) encouraging the reduction-oxidation of inorganic P compounds (Alexander 1977). With PSB, Premono (1994) found that P derived from fertilizer and fertilizer use efficiency increased as much as 60-135%. Andriani (1997) reported that the use of PSB increased P availability and nutrient uptake of corn in acid soils. The mechanism of increasing P availability and absorption due to PSB also occurred in the inoculation of Tithonia with PSB.

The results of bacterial inoculants inoculated into rhizosphere of Tithonia in this field trial seem to be in line with the results of a pot trial in the greenhouse (Asman *et al.* 2008). They reported that inoculation of PSB or PSB mixed with *Azospirillum* into the rhizosphere of Tithonia resulted in higher N, P, and K than that from a mixture of other bacterial inoculants. Venkateswarlu and Rao (1983) reported that N fixing bacteria such as *Azospirillum* was commonly found in the area around the roots, the root surface and in the root. Berg *et al.* (1980) suggest that *Azospirillum* is able to penetrate the epidermis and the root cortex of plants by forming a capsule fixing atmospheric nitrogen and establishing a close association with its host. Beside N fixation, *Azospirillum* also produced plant growth promoters or phytohormones that are very important for root and above root plant growth.

Based on these experimental results, it could be stated that the PSB inoculation of Tithonia rhizosphere either singly, or in combination, improved uptake of plant

nutrients, produced high DM, and high N, P, and K content. Kloepper *et al.* (2004) and Glick (1995) suggest that the effect of soil microorganisms on plant growth is very important to improve productivity of plants and maintain soil fertility.

CONCLUSION

Phosphate-solubilizing bacteria, and/or its combination with *Azospirillum* (N-fixing bacteria) were found to be the most suitable bacterial inoculants for cultivating Tithonia as hedgerow in Ultisols to obtain high dry matter yield and high N, P, and K content. Inoculation of phosphate-solubilizing bacteria into Tithonia rhizosphere produced 11.3 t dry matter, 215 kg N; 30 Kg P, and 253 kg K/2000 m² ha⁻¹ y⁻¹, whereas the-phosphate solubilizing bacteria+ *Azospirillum* inoculation produced about 7.6 t dry matter, 225 kg N, 30 kg P, and 142 kg K/2000 m² ha⁻¹ y⁻¹. The phosphate-solubilizing bacteria (PSB) and /or PSB combined with *Azospirillum* bacterial inoculants is recommended as bacterial inoculant for cultivating Tithonia diversifolia as hedgerow for production of high in situ organic fertilizer in Ultisols.

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