

The Second International Conference on Genetic Resources and Biotechnology

**Harnessing Technology for Conservation and Sustainable
Use of Genetic Resources for Food and Agriculture**

Bogor, Indonesia • 24–25 May 2021

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January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES
AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable
Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

Cite as: AIP Conference Proceedings **2462**, 010002 (2022); <https://doi.org/10.1063/12.0008934>
Published Online: 19 January 2022



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Published Online: 19 January 2022



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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021 virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Conference date: 24–25 May 2021

Location: Bogor, Indonesia

ISBN: 978-0-7354-4172-9

Editors:

I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tru Puji Priyanto, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan

Volume number: 2462

Published: Jan 19, 2022

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ISBN: 978-0-7354-4172-9

Editors:

I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tru Puji Priyanto, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan

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Cite as: AIP Conference Proceedings **2462**, 060002 (2022); <https://doi.org/10.1063/5.0075503>
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Abstract. An efficient cultivation procedure is required to support the production of artemisinin from *Artemisia annua* L. As a secondary plant metabolite, artemisinin production is influenced by environmental factors such as the availability of nutrients. This research aimed to evaluate nutrient use efficiency (NUE) of N, P, and K and biofertilizer effectiveness in *A. annua* cultivation under treatments of various rates of nutrients provided in relation to artemisinin production. Leaves dry mass, leaves nutrients concentration (N, P, and K), artemisinin content, and artemisinin yield were measured to analyse NUE. Four indices of NUE, i.e. partial factor productivity (PFP), apparent crop recovery efficiency (RE), physiological efficiency (PE), and agronomic efficiency (AE), were used for evaluation. The result showed that the biofertilizer increases the artemisinin content, artemisinin yield, and NUE. Two of the four indices, PFP and AE, show the significant role of biofertilizer on NUE. The NUE level of plants with biofertilizer treatment increased 2.2 and 4.5 times than plants without biofertilizer. Therefore, it is recommended to use biofertilizer in *A. annua* cultivation for artemisinin production.

INTRODUCTION

Artemisia annua L. is the major source of artemisinin, a chemical compound that is used in antimalarial therapy [1]. This plant was known as traditional medicine in China which then becomes popular as antimalarial medicine after resistances to quinin was found. WHO recommended artemisinin-based therapy to cope the resistances [2]. This plant contains approximately 0.1 to 0.8% of artemisinin [3]. Although this plant originally occurs on subtropical climate, some cultivation experiments have shown that it also could grow on tropical climate [4]. Gusmaini and Nurhayati [5] reviewed that *A. annua* is promising medicinal plant to cultivate in Indonesia to support national self-sufficiency of basic drugs materials.

Artemisinin is one of secondary metabolites of *A. annua*. It was mostly extracted from leaves of *A. annua* [1]. However, the glandular trichomes, where the artemisinin produced, were also found on flowers and trunk of *A. annua* [6, 7]. Leaf characteristics are important traits as a proxy of artemisinin yield [8]. Artemisinin yield has been reported to increase through enhancement of leaf biomass which is affected by fertilizer addition [3, 9–11]. The leaf biomass and artemisinin yield of *A. annua* are reported to have positive relationship with nutrients (nitrogen, phosphorus, and potassium) availability [9–11]. However, it was also found that the artemisinin content is negatively correlated to nutrient provided [9, 10]. It is important to address for measurement of optimum fertilizer

rates since fertilization takes a big portion of plant production cost, but on the other hand, excessive rates of fertilization had negative impact to the environment [12].

The ability of plants or cultivars to absorb the nutrient and use it for production of plant biomass and/or other products is known as nutrient use efficiency (NUE) [13]. It was estimated that the fertilization efficiency in tropical area was less than 50% for N, less than 10% for P, and close to 40% for K [14]. Therefore, some experiments are required to increase the NUE. NUE is related to soil capacity to supply adequate nutrients and plant ability to acquire, transport, and remobilized nutrient to all parts of plant [13, 15]. There are many factors that influence the NUE, such as soil factors, types of fertilizer, plant species, plant genetic, cultivation techniques, climate, and plant-soil microbes association [13]. By modifying those factors through using efficient plant genotypes, efficient cultivation techniques, optimum fertilizer rates or application of biofertilizer which contain symbiotic soil microbes, the NUE could be enhanced.

In terms of types of fertilizer, organic fertilizer was reported to be beneficial on growth of *A. annua* and production of artemisinin as well as the inorganic fertilizer [16, 17]. Hartatik and Widowati [18] stated that organic fertilizer such as manure only contains small amount of nutrients for plant, and therefore, they stated that the application of organic fertilizer is more addressed to improve biological and physical properties of soil. In rosemary cultivation, Singh [19] reported that additional of organic material increased the NUE of the plant.

Symbiotic soil microbes, such as mycorrhizal fungi [11, 20, 21] and endophytic bacteria [22], have been reported to influence artemisinin content in *A. annua*. Mandal *et al.* [21] reported that mycorrhizal plant of *A. annua* without additional P-fertilizer has similar artemisinin content and trichome density with non-mycorrhizal plant with additional P-fertilizer and methyl jasmonate. However, there is no report on the magnitude of the effectiveness of inoculation of symbiotic soil microbes on different nutrients provided in relation of artemisinin production from *A. annua*. This research aimed to evaluate NUE of N, P, and K and biofertilizer effectiveness in *A. annua* cultivation under treatments of various rates of nutrients provided in relation to artemisinin production.

MATERIALS AND METHODS

All seeds were sown on sterilized compost. One month after sowing, seedlings were transplanted into individual plastic pots. Seedlings were divided into two groups: with and without biofertilizer application. The biofertilizer, *Miza Plus* produced by Indonesian Research Institute for Biotechnology and Bioindustri (IRIBB), contains spore of mycorrhiza of *Acaulospora tuberculata* and three non-symbiotic soil bacteria (N-fixing bacteria, *Pseudomonas* sp. and *Serratia marcescens*). The inoculant has mycorrhiza spore density about 10^5 spores per g whilst there is no available information of the density of the non-symbiotic bacteria in the *Miza Plus* product. After 2 weeks within individual pots, seedlings were transplanted into 40 kg plastic bags of soil placed in the field. Plant densities were 40,000 individuals per ha. The growing medium was soil only or with addition of horse dung manure at the rate of 15 t/ha. Based on Hartatik and Widowati [18], horse dung contains 0.5% of N, 0.25% of P, and 0.3% of K.

The experiments were set using a split-split plot design with three factors. The first factor was the application of four different rates of inorganic fertilizer NPK (0 kg/ha, 40:40:40 kg/ha, 80:80:80 kg/ha, and 120:120:120 kg/ha). The inorganic fertilizer NPK was applied in the form of urea for N, SP-36 for P, and KCl for K. The second factor was manure addition (with and without 150 t/ha). The third factor was biofertilizer (with and without 15 g per plant of *Miza Plus*). Each treatment has ten replications. By considering the proportion of NPK in manure and inorganic fertilizer, total of N, P, and K provided per plant was calculated (Table 1).

TABLE 1. Calculation of total N, P, and K provided per plant.

No.	Treatment	Total N/P/K provided (g) per plant		
		N	P	K
1	Without manure and NPK	0.00	0.00	0.00
2	Manure only	1.88	0.94	1.13
3	40 kg/ha N	2.17	2.78	1.67
4	Manure + 40 kg/ha N	4.05	3.72	2.79
5	80 kg/ha N	4.35	5.56	3.33
6	Manure + 80 kg/ha N	6.22	6.49	4.46
7	120 kg/ha N	6.52	8.33	5.00
8	Manure + 120 kg/ha N	8.40	9.27	6.13

Leaf biomass was weighted after harvest. Harvesting time was just before the plant start to flowering at around 3 months after planting. Leaves were dried at 40°C for 48 h prior to weight for dry biomass and then sent for nutrient content analysis and artemisinin analysis.

Leaf nutrient content analyses were conducted using wet destruction methods. N, P, and K content of leaves were determined by N-Kjedal method, Olsen or Bray method, and Morgan method, respectively. The leaf nutrient uptake (g per plant) was calculated as multiplication of leaf nutrient content (%) to total leaves dry mass per plant (g per plant). Nutrient uptake efficiency was calculated as a result of NPK uptake/NPK applied $\times 100\%$.

Artemisinin content was analysed based on the weight of dried leaves. Maceration technique of methanol for 24 h per sample was used for extraction. The extracts were filtrated and dried under vacuum condition prior to weighing. Organic solvents, such as n-hexane, ethyl acetate, and methanol were used for partitioning and drying the extracts before being analysed by HPLC. Analysis of artemisinin content was conducted using Shimadzu HPLC coupled with a UV-detector at 214 nm. Isocratic condition of 1 ml/min of acetonitrile 60% was applied into reverse phase C-18 column. Artemisinin content were quantified using calibration curve of artemisinin standard at a concentration of 125, 250, 500, 1,000 ppm, respectively. Artemisinin yield were calculated by multiplied artemisinin content with leaf dry mass.

NUE was measured using four indices [12], i.e. partial factor productivity (PFP), apparent crop recovery efficiency (RE), physiological efficiency (PE), and agronomic efficiency (AE). PFP aims to evaluate the productivity of the cropping system in relation to nutrient input. RE indicates the ability of crop to take up the applied nutrient from the soil. PE indicates the transformation efficiency of nutrient acquired into crop yield. Whilst, AE is used to define the efficiency of economic yield increments per unit inputs. On the measurement of NUE, the control plants (plants without NPK fertilizer, manure, and biofertilizer) were performed as a base for comparison. The formula for the calculation of these four indices (%) was:

$$PFP = (Y / N) \times 100\%$$

$$RE = ([U - \overline{Uo}] / N) \times 100\%$$

$$PE = ([Y - \overline{Yo}] / [U - \overline{Uo}]) \times 100\%$$

$$AE = ([Y - \overline{Yo}] / N) \times 100\%$$

where N = amount of nutrient provided (g per plant), Y = artemisinin yield in plants treated with fertilizer and/or without manure, $\overline{Yo}$ = mean of artemisinin yield in leaves of control plants, U = total nutrient uptake in leaves of plants treated with fertilizer and/or without manure, and $\overline{Uo}$ = total nutrient uptake in leaves of control plants.

Data were tabulated and analysed using IBM® SPSS® version 21 for statistical analysis of variance (ANOVA). Leaf dry biomass, artemisinin content, artemisinin yield, nutrient uptake, nutrient uptake efficiency, PFP, RE, PE, and AE were plotted on diagrams to show the differences between plant with and without biofertilizer.

RESULTS AND DISCUSSIONS

Leaf Dry Mass, Artemisinin Content, and Artemisinin Yield of *Artemisia annua* L.

The result shows that the leaf biomass of *A. annua* was only affected by addition of NPK fertilizer (Table 2). On average, the lowest leaf dry mass (25.46 g per plant) was found on control plant without fertilization. On the other hand, the highest leaf dry mass (64.86 g per plant) was found on plant with treatment of NPK fertilizer with doses 120:120:120 kg/ha (Table 3). Manure and biofertilizer addition had no effect to leaf dry mass. Leaf dry mass increased by the increasing rates of NPK fertilizer (Fig. 1a).

Artemisinin content and artemisinin yield were affected by interaction effect of NPK fertilizer, manure, and biofertilizer (Table 2). The highest artemisinin content which reached up to 0.29% was found on plants with treatment of manure and biofertilizer only (Table 3). On the other hand, the highest artemisinin yield which reached up to 0.094 g per plant or 3.76 kg/ha was found on plants received NPK (120:120:120 kg/ha), manure, and biofertilizer treatments (Table 3). In general, the enhancement of NPK doses decreased the artemisinin content, whereas plant treated with biofertilizer produced more artemisinin (Figs. 1b and 1c).

TABLE 2. Analysis of variance of effect of NPK fertilizer (F), manure (M), and biofertilizer (B) applications and their interactions on leaf dry biomass, artemisinin content, nutrient uptake, nutrient uptake efficiency, partial factor productivity, recovery efficiency, physiological efficiency, and agronomic efficiency of *Artemisia annua* L.

Parameter	Effect						
	NPK	Manure	Biofertilizer	Interaction			
	F	M	B	F x M	F x B	M x B	F x M x B
Leaves dry mass (g per plant)	***	ns	ns	ns	ns	ns	ns
Artemisinin content (%)	***	***	***	***	ns	**	***
Artemisinin yield (g per plant)	***	***	***	***	ns	**	***
Nutrient uptake (g per plant):							
N	***	ns	ns	ns	ns	ns	ns
P	***	***	ns	*	ns	ns	ns
K	***	**	ns	*	*	*	ns
Nutrient uptake efficiency (%):							
N	***	***	ns	**	ns	ns	ns
P	***	ns	ns	ns	ns	ns	ns
K	***	**	ns	*	ns	ns	ns
Partial factor productivity (%):							
N	***	*	***	**	**	ns	ns
P	***	ns	***	ns	***	ns	ns
K	***	*	***	*	***	ns	ns
Recovery efficiency (%):							
N	ns	**	ns	ns	ns	ns	ns
P	***	ns	ns	ns	ns	ns	ns
K	ns	ns	ns	ns	ns	ns	ns
Physiological efficiency (%):							
N	ns	ns	ns	ns	ns	ns	ns
P	ns	ns	ns	ns	ns	ns	ns
K	ns	ns	ns	ns	ns	ns	ns
Agronomic efficiency (%):							
N	***	ns	***	ns	***	ns	ns
P	***	ns	***	ns	***	ns	ns
K	***	ns	***	ns	***	ns	ns

***Significant at $p < 0.001$. **Significant at $p < 0.01$. *Significant at $p < 0.05$. ns = not significant.

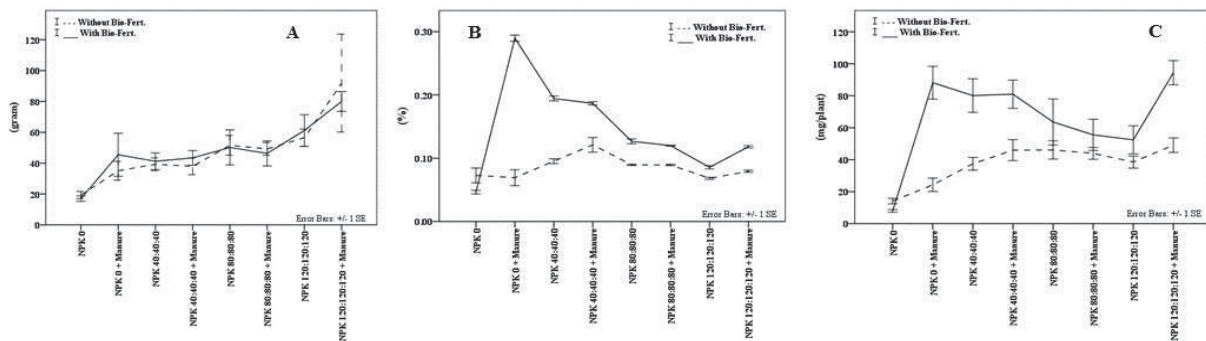


FIGURE 1. Leaf dry mass (a), artemisinin content (b), and artemisinin yield (c) of *Artemisia annua* L. under treatments of NPK fertilizer at different doses and with and without the application of manure and biofertilizer. Solid line (—) refers to plants with treatment biofertilizer and dash line (---) refers to plants without biofertilizer.

TABLE 3. Means of leaf dry mass, artemisinin content, artemisinin yield, nutrient uptake, and nutrient uptake efficiency of *Artemisia annua* L. based on the main effect or the interaction effects under treatments of NPK fertilizer, manure, and biofertilizer.

Treatment			Leaf dry mass (g/ plant)	Artemisinin content (%)	Artemisinin yield (g/ plant)	Nutrient uptake (g/plant)			Uptake efficiency (%)		
						N	P	K	N	P	K
F	M	B	F̄	F x M x B	F x M x B	F̄	F x M	F x M	F x M	F̄	F x M
0:0:0	-	-	25.46 a	0.073 bc	0.014 a	1.108 a	0.032 a	0.52 a	-	9.222 c	-
	-	+		0.046 a	0.008 a						
	+	-		0.069 b	0.024 ab						
	+	+		0.290 g	0.088 f						
40:40:40	-	-	40.46 b	0.095 d	0.037 bc	1.746 b	0.098 b	0.958 b	82.362 c	3.021 b	58.193 b
	-	+		0.194 f	0.080 ef						
	+	-		0.121 e	0.046 bcd						
	+	+		0.187 f	0.081 ef						
60:60:60	-	-	49.31 c	0.090 cd	0.046 bcd	2.307 c	0.094 b	1.132 b	55.38 b	1.727 a	33.969 a
	-	+		0.127 e	0.064 de						
	+	-		0.089 cd	0.044 bcd						
	+	+		0.120 e	0.056 cd						
120:120:120	-	-	64.86 d	0.068 b	0.038 bc	2.999 d	0.128 c	1.494 c	42.428 ab	1.689 a	29.882 a
	-	+		0.086 bcd	0.053 cd						
	+	-		0.079 bcd	0.049 cd						
	+	+		0.118 e	0.094 f						

Number followed by same letter in the same column means not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$. F = fertilizer, M = manure, B = biofertilizer

Nutrient Uptake and Nutrient Use Efficiency (NUE)

Nutrient uptake and NUE were not affected by biofertilizer application (Table 2). N uptake was only affected by NPK fertilizer, while P and K uptakes were much affected by the interaction of NPK fertilizer and manure. The addition of manure increased the uptake of P and K (Table 3). These show that manure increases the availability of P and K. Enhancement of nutrients provided by NPK fertilizer increased an uptake of N, P, and K (Fig. 2a, 2b, and 2c). The highest NPK uptake was found on plants with NPK fertilizer at dose 120:120:120 kg/ha treatment and manure + biofertilizer treatment.

Application of biofertilizer had no significant effect to nutrient uptake efficiency (Table 2). The addition of manure decreases the N- and K-uptake efficiency but had no effect on P-uptake efficiency (Table 3). Nutrients (N, P, and K) uptake efficiency decreased by the increasing rates of nutrients provided (Figs. 3a, 3b, and 3c).

In general, based on PFP and AE, NUE decreased by the increasing doses of nutrients provided (Table 2). Table 4, Figs. 3d and 3e, and Figs. 3m, 3n, and 3o show a significant role of biofertilizer to NUE. On average, biofertilizer increased 2.2 times higher and 4.5 times higher of NUE (N, P, and K) based on PFP and AE values. Apparent RE show different pattern for N, P, and K (Figs. 3g, 3h, and 3i). Nitrogen RE was more affected by manure addition (Table 2). Plants treated with manure addition have less RE of N than plants without manure (Fig. 3g). RE of P was much affected by NPK fertilizer. Increasing rates of P provided had decreased RE of P (Table 4, Fig. 3h). RE of K were not influenced by any treatment factors. PE did not show significant differences of NUE for N, P, and K in all the treatments (Figs. 3j, 3k, and 3l).

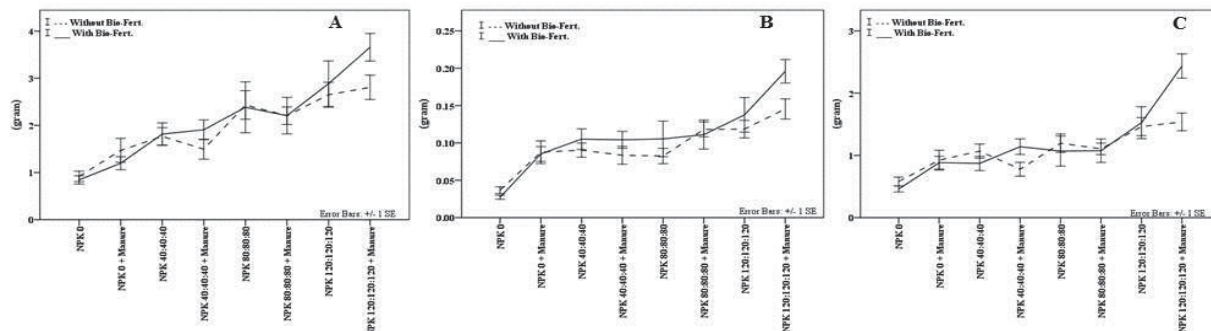


FIGURE 2. Uptakes of nitrogen (a), phosphorus (b), and potassium (c) of *Artemisia annua* L. under treatments of NPK fertilizer at different doses, and with and without the application of manure and biofertilizer. Solid line (—) refers to plants with treatment biofertilizer and dash line (---) refers to plants without biofertilizer.

TABLE 4. Means of nutrient use efficiency (NUE) based on apparent crop recovery efficiency, partial factor productivity, and agronomic efficiency indices of *Artemisia annua* L. by the main effect or the interaction effects under treatments of NPK fertilizer, manure, and biofertilizer.

Treatment			Recovery efficiency (%)	Partial factor productivity (%)			Agronomic efficiency (%)		
NPK	Manure	Bio-fertilizer	P	N	P	K	N	P	K
F	M	B	F	F x B	F x B	F x B	F x B	F x B	F x B
0:0:0	-	-	5.274 b	1.294 b	2.59 b	2.157 c	0.548 a	1.096 a	0.913 a
	+	-		4.701 d	9.406 c	7.835 e	3.954 c	7.913 c	6.59 c
	+	+		1.429 b	1.293 a	1.947 bc	0.934 a	0.852 a	1.276 a
40:40:40	-	-	1.857 a	2.842 c	2.531 b	3.853 d	2.347 b	2.091 b	3.182 b
	+	-		0.883 ab	0.753 a	1.184 ab	0.609 a	0.519 a	0.817 a
	+	+		1.177 ab	0.999 a	1.576 abc	0.904 a	0.766 a	1.209 a
60:60:60	-	-	1.109 a	0.588 a	0.496 a	0.786 a	0.397 a	0.336 a	0.532 a
	+	-		0.964 ab	0.824 a	1.295 abc	0.773 a	0.664 a	1.04 a
	+	+							
120:120:120	-	-	1.267 a						
	+	-							
	+	+							

Number followed by same letter in the same column means not significantly different according to Duncan's Multiple Range Test at $\alpha = 0.05$.

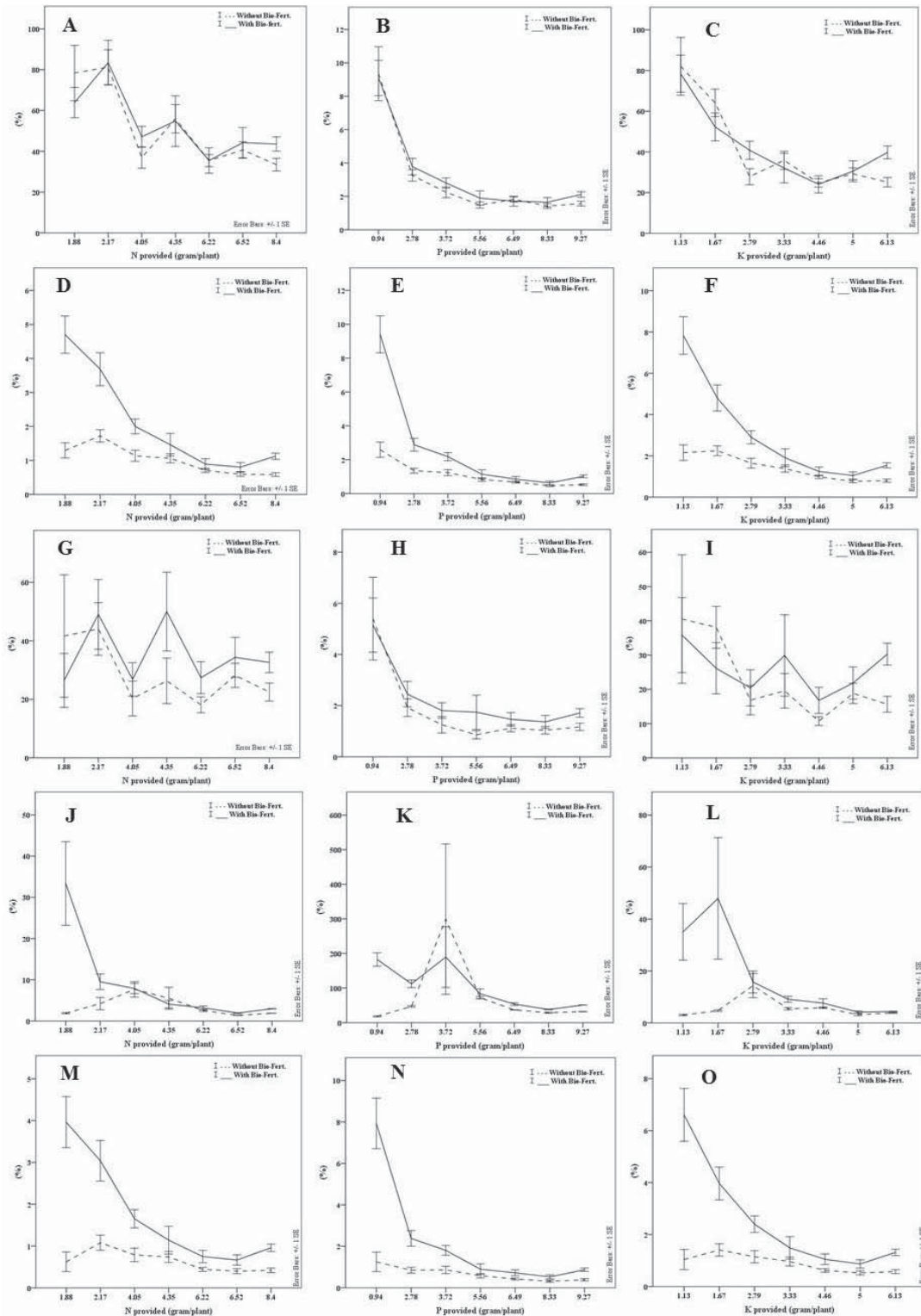


FIGURE 3. Nutrient uptake efficiency (NUE) of N (a), P (b), and K (c); partial factor productivity (PFP) of N (d), P (e), and K (f); apparent crop recovery efficiency (RE) of N (g), P (h), and K (i); physiological efficiency (PE) of N (j), P (k), and K (l); agronomic efficiency (AE) of N (m), P (n), and K (o); at various rates of nutrients provided under treatments of NPK fertilizer at different doses and with and without the application of manure and biofertilizer. Solid line (—) refers to plants with treatment biofertilizer and dash line (---) refers to plants without biofertilizer.

The results show an interaction effect of NPK fertilizer, manure, and biofertilizer application on artemisinin production of *A. annua*. NPK fertilizer and manure increased the nutrient provided for the plants. However, only NPK fertilizer application significantly increased the leaf dry mass. The highest artemisinin content was found in plants without NPK fertilizer but with manure and biofertilizer addition. At higher dose of NPK fertilizer, the artemisinin content was low, but the application of biofertilizer could increase it. It seems that nutrient stress induced the production of artemisinin. Manure addition significantly increased artemisinin content and artemisinin yield when biofertilizer added. P- and K-uptake also increased when manure was added. This finding is similar to the results of Jha and collaborators [16]. They found that manure application increases artemisinin yield of *A. annua* as well as inorganic fertilizer application. They also stated that manure also improves the biological properties of soil. Biofertilizer has increased artemisinin content, artemisinin yield, and nutrient use efficiency based on PFP and AE.

The important role of biofertilizer on production of artemisinin in *A. annua* cultivation has been reported and elaborated by various authors [11, 20, 21, 23, 24]. The role of biofertilizer to artemisinin content elevation can be explained by four mechanisms. Firstly, biofertilizer could increase the density of glandular trichomes, where artemisinin produced [11]. Secondly, biofertilizer could improve plant nutrition uptake on *A. annua* [20], where many of plant nutrients become available and take part as precursors on artemisinin biosynthesis. Thirdly, biofertilizer could induce increasing levels of reactive oxygen species (ROS) in *A. annua* which then in metabolic system scavenged by an antioxidant, and then stimulate artemisinin production [20, 25]. Lastly, biofertilizer could stimulate artemisinin biosynthesis by increasing jasmonate content in *A. annua* [21, 26]. The current result shows that at the same levels of nutrient uptake, plants with biofertilizer treatment produced more artemisinin (Fig. 1b). It means that the plants with biofertilizer use the nutrients more efficient to produce artemisinin. The result also shows that RE and PE, as the other NUE indices, are not affected by the treatments. Those two indices are probably cannot be used for measurement at leaf level, but for whole the plants [13].

Based on the result, the production of artemisinin of *A. annua* can be optimum when biofertilizer applied. Addition of NPK fertilizer and/or organic fertilizer (manure) is required to prevent soil fertility depletion and promote a sustainable cultivation. On the other hand, addition of high rates of NPK fertilizer was not suggested because it is not economically satisfied due to low artemisinin content and could cause environmental hazard. The balance input or the optimum input for artemisinin production should be examined in the cultivation area of *A. annua* to get more accurate data of soil nutrient capacity for specific cultivation area.

CONCLUSION

NPK fertilizer and manure increased the nutrient provided for plants. Leaf dry mass and nutrients (N, P, and K) uptake increased with increasing nutrients provided but the artemisinin content and NUE were in contrast. Biofertilizer application increased artemisinin content, artemisinin yield, and NUE. The NUE level of plants with biofertilizer application increased 2.2 and 4.5 times higher than plants without biofertilizer based on PFP and AE indexes, respectively. Thus, at the same rate of nutrients provided, plants with biofertilizer can produce more artemisinin.

ACKNOWLEDGMENTS

We highly appreciated Mr. Latif for field assistance during the field experiment and observation.

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