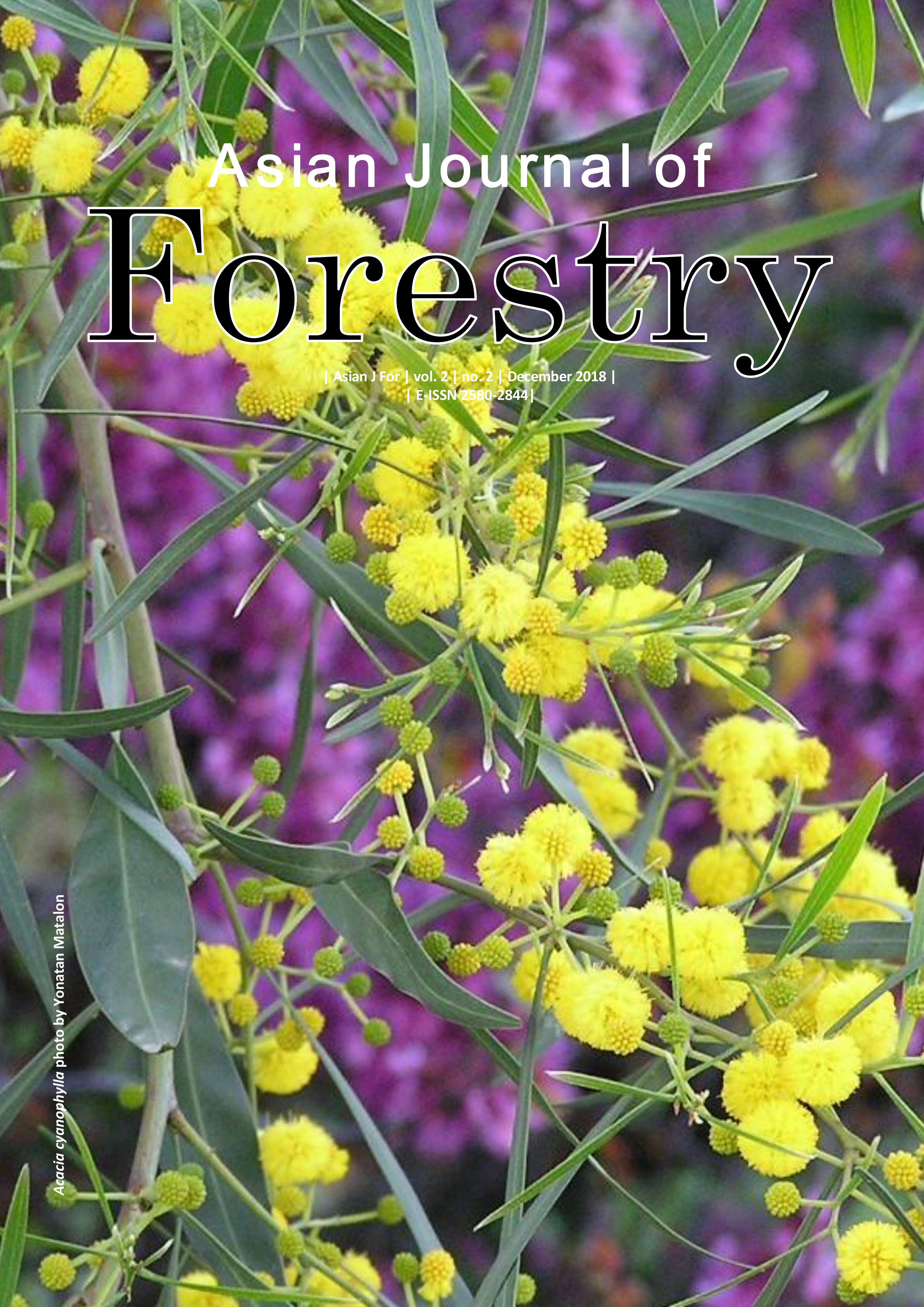

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Acacia cyanophylla photo by Yonatan Matalon

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Figures and Tables of maximum of three pages should be clearly presented. Title of a picture is written down below the picture, while title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; chart is preferred to use black and white images. Author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned source. **There is no appendix**, all data or data analysis are incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

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Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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Effect of symbiotic association of rhizobia and endomycorrhizae from Moroccan arid littoral dunes on *Acacia cyanophylla* tolerance to drought

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Abstract. *Hatimi A, Tahrouch S, Bouizgarne B. 2018. Effect of symbiotic association of rhizobia and endomycorrhizae from Moroccan arid littoral dunes on* Acacia cyanophylla *tolerance to drought. Asian J For 2: 39-45.* Coastal sand dunes in arid region serve as habitat of coastal biotas including rhizobia and mycorrhiza. This research aimed to investigate the effect of selected symbiotic rhizobia native from coastal dunes of Souss-Massa, Morocco, alone or in association with endomycorrhiza to improve the tolerance of *Acacia cyanophylla* plants to drought stress. A symbiotic indigenous endomycorrhizal fungi M, and three rhizobia isolates (i.e., two slow-growing isolates R1 (*Bradyrhizobium* sp. RCM6), and R2 (*Bradyrhizobium* sp. RLC3) and a fast-growing isolates R3 (*Rhizobium* sp. S21)) were inoculated to *A. cyanophylla* under drought stress conditions in greenhouse. Results showed that the growth and nutrition of seedlings of *A. cyanophylla* were drastically affected after two months in drought stress conditions. However, inoculation of the symbiotic microorganisms either alone (treatments M, RMC6, R2 or R3) or as inoculums consisting of combination of the rhizobia with the endomycorrhiza (treatments MR1, MR2 or MR3) resulted in enhanced tolerance of *A. cyanophylla* seedlings to drought stress. At 100% of field capacity (fc), all treatments showed a significant improvement of plant growth compared to non-inoculated plants in stress conditions. In addition, *Bradyrhizobium* RCM6 (R1) holds a high efficiency to improve the growth and nutrition of the host plant. Indeed, higher number of nodules/plant and higher amount of total nitrogen were recorded in the seedlings inoculated with *Bradyrhizobium* sp. RCM6 in comparison with plants inoculated with the two other rhizobia *Bradyrhizobium* sp. RLC3 (R2) and *Rhizobium* sp. S21 (R3), and control plants. Dual inoculation with each of the three rhizobia and the endomycorrhizal complex (M) led to higher water content (WC) and relative water content (RWC) and a significant increase in Phosphorus content of the aerial part. While positive effects were recorded for Phosphorus, no such effects were recorded for nitrogen. However, the overall results showed the importance of the use of microorganisms in the dune coastal environment particularly adequate tripartite association: rhizobia Endomycorrhizes-*A. cyanophylla* in enhancing tolerance to drought stress.

Keywords: *Acacia cyanophylla*, endomycorrhizae, plant growth and nutrition, *Rhizobia*, drought stress

INTRODUCTION

Coastal dunes are characterized by the presence of vegetation, which plays an important role in the fixation of sand to form dunes. These dunes are very beneficial to mitigate beach abrasion, storm avoidance and habitat of coastal biotas including rhizobia and mycorrhiza.

Coastal dunes in Sous-Massa, South-West of Morocco have a very low vegetation cover dominated by two legumes (*Retama monosperma* and *Ononis natrix*) and Gramineae species *Schismus barbatus* (Hatimi and Tahrouch, 2007). In this region, *Acacia cyanophylla* is used for the fight against desertification, coastal dune fixation, forage production and wood supply.

In the coastal region of Souss-Massa, drought is the main constraint to crop production (Sanchez-Diaz et al. 1995). This arid area is known for low rainfall and high temperatures that lead to increased soil salinity. Simultaneously, such climate and soil factors have adverse effects on plants due to limited water availability and nutrient losses. Water stress conditions can also influence soil microflora, for example, it could disturb the symbiotic microorganisms of rhizobia and endomycorrhiza.

In general, drought stress affects the degree of legumes infection by rhizobia (Mnasri et al. 2007) and N_2 fixation in legumes (Marques et al. 2001; Mnasri et al. 2007; Gong et al. 2013). *Acacia* plants could undergo deleterious drought effects in arid areas. However, the role of *Acacia* as biological dune fixation is limited by the lack of water. Many studies indicate that it is possible to select rhizobia strains resistant to drought and showing also high nitrogen-fixation effectiveness (Shoushtasi and Papper 1985a, 1985b; Romdhane et al. 2008, 2009).

In addition to nitrogen, the beneficial roles of mycorrhizal fungi are proved to be relevant in providing better phosphorus supply to plants (Barea et al. 2005; Wu et al. 2007; Evelin et al. 2012), to induce changes in water relations of plants (Marulanda et al. 2007; Porcel and Ruiz-Lozano 2004; Ruiz-Lozano and Aroca 2006; Bárzana et al. 2012), to induce higher water conductivities and more effective water uptake (Fagbola et al. 2001; Augé 2001; Aroca et al. 2007) and to increase in growth rate and biomass (Porcel and Ruiz-Lozano 2004; Wu et al. 2008). Many kinds of research also noted the importance of using tripartite association legume-rhizobia-mycorrhizal symbiosis as efficient system in harsh conditions (Marques et al. 2001; Ruiz-Lozano et al. 2001; Porcel and Ruiz-Lozano 2004; Barea et al. 2005; Aroca et al. 2007; Sprent and James 2007; Bouizgarne et al. 2015). In this framework, the aims of this research were to investigate the effect of selected symbiotic rhizobia, alone or in association with endomycorrhiza to improve the tolerance of *A. cyanophylla* plants to drought stress

MATERIALS AND METHODS

Plant materials

The study focused on *Acacia cyanophylla*, a leguminous tree originating from Australia. The plant has a particularly deep root system, rapid growth and great adaptation to the dune environment. *Acacia cyanophylla* is useful to fix both the dune sands and the ravines banks, and is one of the basic materials in forest grazing areas due to the feed value of its leaves and pods. For these reasons, Water and Forest Service of the Government of Morocco introduced this species in order to increase the biological stabilization of coastal dunes in Souss-Massa region in the southern part of Agadir Province, South of Morocco.

Four inocula those were A mycorhizogen complex (M), consisting of a consortium of endomycorrhizal fungi obtained from the dune soil from Lamzar region with high colonization potential of *Allium porrum* roots (Plenchette et al. 1989). Three isolates of rhizobia: two isolates of the slow-growing *Bradyrhizobium: Bradyrhizobium* sp., RCM6 (R1), RLC3 (R2) and one isolate of fast-growing *Rhizobium* sp., S21 (R3) isolated from the nodules of *Acacia cyanophylla* collected from three different areas of the high saline coastal dunes of Souss-Massa.

Methods

Cultures

Seeds of *Acacia cyanophylla* were scarified and disinfected through one-hour immersion in concentrated sulfuric acid. Subsequently, they were allowed to germinate for 7 days in sterile vermiculite. Seedlings of the same size were then transplanted into pots (two seedlings per pot). Each pot contained 2 kg of a 1: 1 (w/w) sand-vermiculite mixture which was previously washed vigorously and autoclaved twice at 120 °C for 1 hour. The pots were then transferred to greenhouse, maintained at temperatures of approximately 28°C at day and 20°C at night and 12 hours photoperiod after 7 days of culture. Treatments consisting of inoculation of seedlings with each of the three rhizobia *Bradyrhizobium* spRCM6 (R1), *Bradyrhizobium* sp. RLC3(R2) and *Rhizobium* sp, S21 (R3) either alone or in combination with the mycorrhiza mixture (M) were performed: plants were inoculated with 1 ml of a rhizobial suspension at 10^8 cells. ml^{-1} according to Ames and Bergman 1984 or 1 g of the mycorrhized leek roots according to Plenchette et al. (1989). Dual inoculations consisted of 10^8 cells. ml^{-1} rhizobial cells and 1 g of the mycorrhized leek roots. Seven inoculation treatments were applied: R1, R2, R3, M, R1+M, R2+M and R3+M and twelve seedlings were used for each inoculation. Uninoculated plants served as the control.

Drought stress was applied three weeks after inoculation by microorganisms in order to allow time for the establishment of symbiosis. Three water regimes were applied to each treatment: 100, 50 and 25% of field capacity (FC). Along with the experiments where seedlings are exposed to drought stress, the pots water content was adjusted to the weight corresponding to each drought stress level by successive weighing and adding the water. Seedlings received a weekly basic nutrient solution according to Marcar et al. (1991). Both mycorrhizal (M) treatment and uninoculated seedlings (control) received 200 mg N L^{-1} as ammonium nitrate in addition to the nutrient solution (Zou et al. 1995). Four replicates of each of the three drought treatments, each consisting of twelve inoculated and uninoculated seedlings were used.

Plant harvest, growth and mineral nutrition

After two months of drought stress, we estimated the growth and mineral nutrition in *A. cyanophylla* seedlings by measuring the growth, water content, degree of root infection and mineral element parameters.

The growth of *A. cyanophylla* was estimated by measuring the length of the plant aerial system and the biomass produced. The latter was measured by determining the fresh and dry weight of shoot and root parts of the plant. The dry weight was obtained after drying shoots and roots separately after incubation for 48 hours at 80°C. Comparison of dry matter production in stressed and unstressed seedlings (Pr %) that provides information on the degree of stress tolerance of the plant was calculated as:

$$Pr (\%) = 100 (SSDW/NSSDW)$$

Where:
SSDW = Dry weight of the stressed seedling
NSSDW = Dry weight of the stressed seedling

Water content (W. C) was determined by the difference between fresh material (FW) and the dry matter (DW) weight and is expressed in grams of water per gram of dry matter. Values represent the mean of four replications and results are presented as mean ± SD.

$$W. C (g\ H2O/g\ MS) = (FW-DW)/ DW$$

Relative water content (RWC) was measured in the last fully developed leaf from the top of the stem (leaf whose growth is complete). RWC is calculated from the formula:

$$RWC (\%) = 100 \times (FW-DW)/(RW-DW)$$

Where:
FW: Fresh weight of the leaf weighed immediately
DW: dry weight after drying for 48 hours the leaf at 80°C.
RW: rehydrated Weight by immersion in distilled water for 18 h at 4 °C in the dark.

Values represent the mean of four replications and results are presented as mean ± SD.

Infection by *Rhizobia* was evaluated by counting the number of nodules formed on the roots (n) and recording their dry weight (DWn) after drying at 80 °C for 48 hours.

The degree of mycorrhizal infection was evaluated by the frequency of infection (F%) according to Trouvelot et al. (1986) below:

$$F(\%) = 100\,(N-No)/N$$

Where:
N: number of root fragments observed (= 20)
No: number of fragments without mycorrhization.

Values represent the mean of four replications and results are presented as mean ± SD.

Total nitrogen (N) of aerial and root parts of the plant was measured by the Kjeldahl method for organic nitrogen digestion and reduction of nitrates eventually present. Total Phosphorus (P) in plant tissue was measured by the nitro-vanado-molybdate method (Jackson, 1958). Potassium content (K) was determined by flame-emission spectrophotometry (type 90D LISABIO PHE) (Rodier 1984). Values represent the mean of four replications and results are presented as mean ± SD.

RESULTS AND DISCUSSION

Effect of symbiont on plant growth

At 100% of field capacity (FC), considered optimal moisture state, plant growth of *A. cyanophylla* associated with root symbionts showed a significant improvement compared with non-inoculated plants. The improvement in growth resulted in the increase in the length of the aerial part of the plant (Figure 1) and in the production of fresh matter of the aerial part of the plant (Figure 2). Increases in the production of dry matter in treatment R1, M and MR1 were 2 to 2. 5 times higher than in the uninoculated control treatment (Table 1). The application of drought stress of 50% or 25% of FC, led to reduced growth in length (Figure 1) and biomass production (Figure 2 and Table 1) in all treatments and we noted that the aerial part of the plant seems to be the most sensitive to drought stress. However, the ratio of dry matter of the stressed plants to that in unstressed plants (100% of FC) was greater in inoculated plants (Table 1). This reflects the tolerance of inoculum complex-host plant to water deficit. This tolerance was very significant (P <0.05) with all endomycorrhiza treatments (M, MR1, MR2, and MR3).

Table 1. Variation of dry matter of the aerial part of the plant (SDW), roots dry matter (RDW), total dry matter (TDW), water content (WC) of the aerial part of the plant and foliar relative water content (RWC) in *A. cyanophylla* inoculated by three isolates of rhizobia RCM6 (R1) RLC3 (R2) and S21 (R3) and /or mycorrhizae complex (M), and exposed to three decreasing water regimes 100%, 50% and 25% of full capacity*.

Treatment	Water regime (% of FC)	SDW (g)	RDW (g)	TDW (g)	Pr** (%)	W. C. (g/g MS)	R. W. C. (%)
Control	100	2.88 ± 0.13	1.05 ± 0.13	3.93 ± 0.25	100	2.84 ± 0.09	90 ± 1.83
	50	0.85 ± 0.17	0.58 ± 0.06	1.43 ± 0.12	36	1.83 ± 0.05	75 ± 3.83
	25	0.56 ± 0.19	0.39 ± 0.07	0.95 ± 0.14	24	1.03 ± 0.06	65 ± 3.16
R1	100	7.52 ± 0.99	1.38 ± 0.25	8.90 ± 0.84	100	4.62 ± 0.27	92 ± 2.94
	50	4.59 ± 0.49	1.06 ± 0.08	5.65 ± 0.51	64	3.98 ± 0.14	85 ± 3.37
	25	3.00 ± 0.22	0.59 ± 0.06	3.59 ± 0.23	44	1.78 ± 0.17	80 ±3.37
R2	100	4.98 ± 0.56	1.38 ± 0.23	6.36 ± 0.52	100	3.90 ± 0.48	93 ± 2.94
	50	3.00 ± 0.15	1.26 ± 0.24	4.26 ± 0.17	67	2.69 ± 0.36	85 ± 3.20
	25	2.32 ± 0.50	0.80 ± 0.07	3.12 ± 0.54	49	1.82 ± 0.19	84 ± 2.87
	100	3.23 ± 0.41	0.54 ± 0.09	3.77 ± 0.43	100	3.41 ± 0.37	90 ± 1.83
R3	50	1.55 ± 0.30	0.36 ± 0.02	1.91 ± 0.29	51	2.82 ± 0.19	85 ± 2.16
	25	1.15 ± 0.13	0.29 ± 0.01	1.43 ± 0.13	38	1.34 ± 0.17	70 ± 3.83
M	100	6.68 ± 0.46	1.04 ± 0.09	7.72 ± 0.44	100	4.24 ± 0.37	94 ± 3.50
	50	4.67 ± 0.14	0.49 ± 0.05	5.16 ± 0.14	67	3.39 ± 0.26	88 ± 1.63
	25	3.37 ± 0.08	0.88 ± 0.10	4.25 ± 0.17	55	2.64 ± 0.20	87 ± 0.82
M+R1	100	6.65 ± 0.29	0.88 ± 0.09	7.53 ± 0.33	100	5.89 ± 0.44	96 ± 2.87
	50	4.58 ± 0.17	0.82 ± 0.11	5.40 ± 0.15	72	4.39 ± 0.18	88 ± 2.71
	25	3.47 ± 0.45	0.27 ± 0.05	3.74 ± 0.49	50	2.88 ± 0.33	85 ± 2.16
M+R2	100	5.75 ± 0.07	0.48 ± 0.10	6.22 ± 0.06	100	4.28 ± 0.32	94 ± 2.63
	50	3.96 ± 0.11	0.68 ± 0.13	4.64 ± 0.16	75	3.97 ± 0.10	90 ± 2.16
	25	2.81 ± 0.11	0.40 ± 0.07	3.21 ± 0.10	52	2.75 ± 0.11	88 ± 1.41
M+R3	100	3.65 ± 0.19	1.16 ± 0.10	4.81 ± 0.27	100	3.33 ± 0.30	93 ± 2.87
	50	2.01 ± 0.14	0.48 ± 0.11	2.49 ± 0.24	52	3.04 ± 0.09	84 ± 2.50
	25	1.47 ± 0.17	0.76 ± 0.15	2.23 ± 0.24	50	2.57 ± 0.15	80 ± 1.83
LSD, *P* = 0,05		0,50	0.18	0.48		0.36	3.83

Note: * FC*: field capacity;** Pr: percentage of total dry matter production by stressed plants in comparison with non stressed plants.

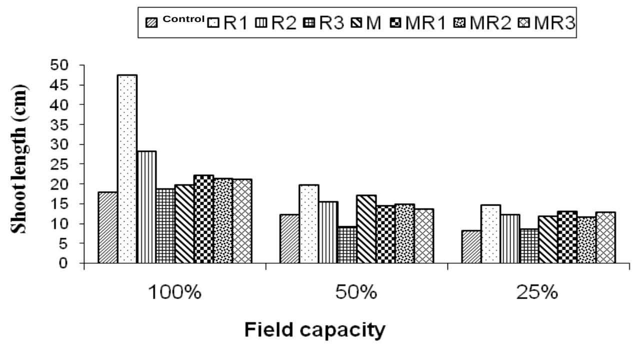

Figure 1. Growth of aerial part of inoculated *A. cyanophylla* plants and subjected to drought stress at 25 and 50 % field capacity. M: Mycorrhizal complex, R1: *Bradyrhizobium* sp., RCM6, R2: *Bradyrhizobium* sp., RLC3 and R3: *Rhizobium* sp., S21.

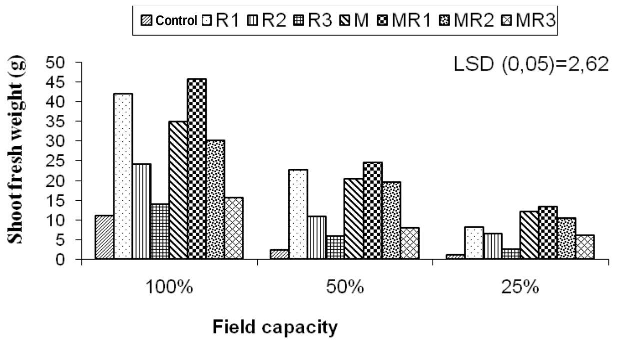

Figure 2. Production of fresh matter in aerial part of inoculated *A. cyanophylla* seedlings and subjected to drought stress. M: Mycorrhizal complex, R1: *Bradyrhizobium* sp., RCM6, R2: *Bradyrhizobium* sp., RLC3, and R3: *Rhizobium* sp., S21.

Water contents

At 100% of field capacity, there has been an increase in the water content (WC) of the aerial part of inoculated plants, ranging from 20 to 100% compared to non-inoculated plants (Table 1). However, in the same water conditions, a slight nonsignificant increase in foliar relative water content (RWC) was observed in plants in symbiotic association. WC of the aerial part of the plant and foliar RWC decreased depending on the degree of applied water deficit. However, the amount of water was always higher in inoculated plants, especially in mycorrhized plants. For instance, at 25% of field capacity, increase in foliar RWC ranged from 7 to 20% in plants inoculated with rhizobia and from 23 to 33% in mycorrhized plants when compared to uninoculated control.

Effect on symbioses: nodular and mycorrhizal infection

The number of nodules per plant was very important in R1 treatment, at 100% of full capacity with 60.5 nodules per plant compared to 46.25 and 32 nodules per plant respectively, in R2 and R3 treatments (Figure 3A). In the same water conditions, the number of formed nodules decreased in the presence of endomycorrhizal fungal mixture (M). Indeed, a reduction of more than 30% in MR1 treatment was observed. Generally, nodules dry weight per plant decreased in the same way as the number of nodules (Figure 3B). Drought stress application of 50% or 25 % of field capacity had a very negative effect on the amount of formed nodules and their dry weight regardless of the treatment used. However, the effect of water deficit on the formation of nodules was strongly reduced with treatment R2. Results also showed that the mycorrhization reduced but in a non-significant way ($P > 0.05$) the effect of stress on the formation of nodules. Figure 3C shows that the difference in mycorrhizal infection degree among treatments and between the different drought stress levels was not significant.

Effect of mineral nutrition

The highest amount of total nitrogen was recorded in the seedlings inoculated with the rhizobia (Fig. 4A). The high concentration of nitrogen was obtained with treatment R1, with 82.87 mg per plant against only 14.55 mg only in the uninoculated plants. At optimal moisture (100% of field capacity), the amount of accumulated total phosphorus was very high among plants inoculated with endomycorrhizal fungi (Figure 4B). These amounts were 4 to 6 times higher than those recorded for the non-inoculated control treatment. A significant increase in the amount of phosphorus was observed in treatments R1 and R2, respectively with 29.94 and 17.31 mg per plant whereas only 11.6 mg per plant was recorded in the non-inoculated treatment. However, in the R3 treatment, the amount of phosphorus was identical to that of non-inoculated plants. The highest concentrations were also observed in mycorrhizal plants. For potassium, large amounts were measured in inoculated plants and especially among mycorrhized plants (Figure 4C). When applying drought stress, the accumulation of total nitrogen in plant tissue decreased dramatically. The potassium content had a similar trend as nitrogen. However, with mycorrhiza, a decrease in the amount of nitrogen was noted and potassium content has reduced while the content of phosphorus varied little with drought stress ($P < 0.05$).

Discussion

Rhizobia are capable of withstanding water potentials lower than a great number of higher plant cells (Sanchez-Diaz et al. 1995). Results obtained in this investigation showed that growth is the first mechanism affected by water deficiency. According to Blanchet et al. (1977), the production of the total dry matter is proportional to the amount of consumed water. At all levels of stress, growth and nutrition are generally greater in inoculated *A. cyanophylla* seedlings when compared with non-inoculated seedlings. The response of root symbionts to drought stress differs from one treatment to another. Indeed, better growth was observed with the strain R1 and a larger tolerance with the strain R2 while strain R3 had no effect on growth and tolerance to drought stress of *A. cyanophylla*. Furthermore, the double inoculation M + R has variable effects according to rhizobia strains. In fact, with this treatment, a reduction in the efficiency of the R1 strain on the growth of *A. cyanophylla* was observed. Similar results were obtained by Lal and Khanna (1993) who worked on the *Glomus-Rhizobium-A. nilotica* association. However, we reached an increased tolerance of the three strains of rhizobia to drought stress when they are associated with the endomycorrhizal complex. In treatments that have shown tolerance to drought stress, we noticed greater water content (WC) and relative water content (RWC) than in non-inoculated control plants. Similar results were obtained in mycorrhized plants subjected to drought stress in *Trifolium* (clover) (Meddich 1997; Meddich et al. 2000), *Lactuca sativa* (Ruiz-Lozano and Azcon 1997) and *Fragaria ananassa* (Hernandez-Sebastia et al. 1999). At 25% of field capacity, RWC was 7-20% and 23-33% higher, respectively, in rhizobia inoculated *A. cyanophylla* seedlings and double inoculated seedlings (R + M). This result highlighted one of the beneficial endomycorrhizal fungi roles which is the great ability to provide water, especially in the case of drought conditions (Aroca et al. 2007, Bárzana et al. 2012).

The water deficiency also affected the degree of legumes infection by rhizobia (Figure 3). In our investigation, the number and dry weight of nodules were affected by drought stress. The reduction was recorded for all treatments. This was observed in many kinds of research. Indeed, establishment of nodulation in the legume-rhizobium symbiosis has long been recognized as being sensitive to drought stress since it is usually associated with a drastic decline in the number of nodules (Soria et al. 1996; Ramos et al. 1999; Sinclair et al. 2001; Mnasri et al. 2007). Therefore, deleterious physiological effects of water deficiency include reduction in nitrogen fixation (Sharma et al. 2010), nodular activity (Sprent 1976; Aparicio-Tejo et al. 1980; Sprent 1981; Serraj et al. 1999) and photosynthesis (Huang et al. 1975), permeability of nodules to oxygen (Aguirreolea and Sanchez-Diaz 1989) and oxidative phosphorylation (Sheehy et al. 1985) have been demonstrated. Unlike infectivity by rhizobia, mycorrhization of *A. cyanophylla* roots was not affected by drought stress. Similar results were obtained by Sanchez-Diaz et al. (1990). The presence of endomycorrhizal fungi

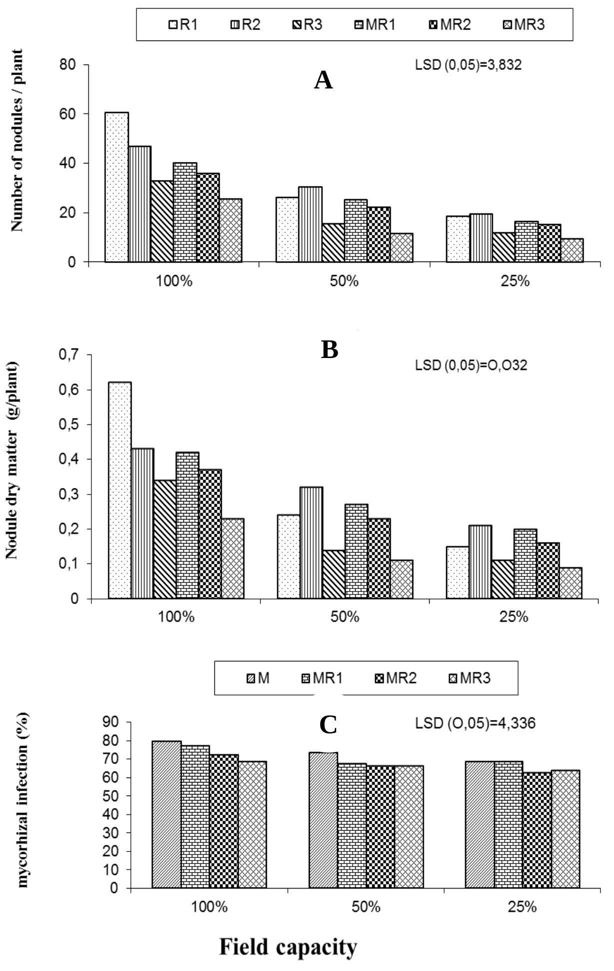

Figure 3. Number of nodules (A), nodules dry matter weight (B) mycorrhizal infection degree (C) of inoculated *A. cyanophylla* seedlings and subjected to drought stress. M: Mycorrhizal complex, R1: *Bradyrhizobium* sp., RCM6, R2: *Bradyrhizobium* sp., RLC3, and R3: *Rhizobium* sp., S21.

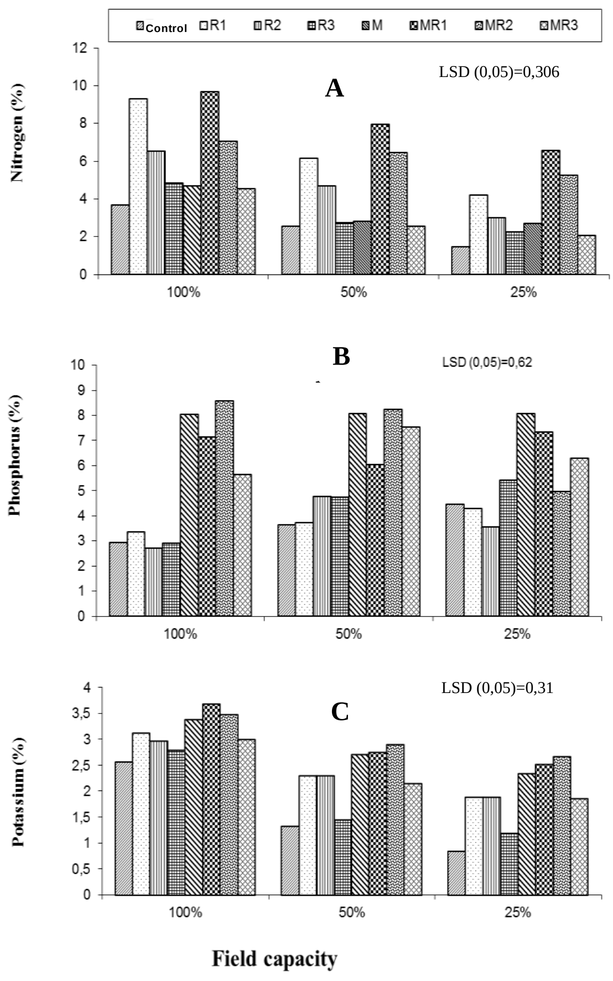

Figure 4. Nitrogen (A), phosphorus (B) and potassium (C) concentration in shoot of inoculated *A. cyanophylla* seedlings and subjected to drought stress. M: Mycorrhizal complex, R1: *Bradyrhizobium* sp., RCM6, R2: *Bradyrhizobium* sp., RLC3, and R3: *Rhizobium* sp., S21.

significantly increased the P content of the aerial part of *A. cyanophylla* (Figure 4A). In general, positive effects of endomycorrhizal fungi on phosphorus uptake have been demonstrated by several authors (Hatimi 1995; Oihabi and Meddich 1996; Barea et al. 2005; Wu et al. 2007; Evelin et al. 2012).

Studies on phosphate nutrition drought-stress relationship in *Acacia* plants are scarce. In our study on *A. cyanophylla,* we obtained an accumulation of P in stressed plants. Indeed, treatments showing some stress tolerance, generally in association with endomycorrhizal fungi, have a higher content of P at the severe drought condition of 25% of field capacity. This high content can be attributed to the degree of infection that was not influenced by the water deficit whereas nitrogen nutrition has not overcome the effects of drought stress. The accumulation of total nitrogen decreased in the tissue of stressed plants even in inoculated plants. Regarding N nutrition, it is well known that the water deficit can act negatively either on the infective capacity of microsymbionts (Mnasri et al. 2007) and the activity of nitrogenase in nodules (Porcel et al. 2003; Naya et al. 2007). In contrast, the effect of stress on the absorption of potassium is relatively reduced when plants are mycorrhized. Indeed, potassium plays a key role in the maintenance of turgor of plant cells (Evelin et al. 2012).

Authors agreed that endomycorrhizal fungi have positive effects on plant photosynthesis and growth (Pena et al. 1988; Sanchez-Diaz et al. 1990; Gong et al. 2013). Sanchez-Diaz et al. (1995) also observed that the extent of mycorrhizal fungi infection was not affected by drought. On the contrary, Meddich et al. (2000) reported that the more drought stress increases, the more the rate of infection of clover (*Trifolium*) by indigenous strains of arbuscular mycorrhiza decreased whereas Goicoechea et al. (1994), observed that alfalfa mycorrhized plants are not affected by drought in their content of cytokinins in comparison with non-mycorrhized plants, suggesting an increase of growth capacity in mycorrhized plants. Although there is growing evidence on the possibility of increased tolerance to drought of mycorrhized legumes due to a better water supply and phosphorus (Berea and Azcòn-Aguilar 1983), it is difficult to distinguish between direct effects of the mycorrhizae and those that could occur through improved nitrogen nutrition by rhizobia.

Results after two months of stress have clearly shown that the growth and nutrition of *A. cyanophylla* seedlings are affected by water deficiency. However, the study has highlighted the importance of inoculation in the tolerance of *A. cyanophylla* plants to stress. Thus, it was shown in this study that the strain of *Bradyrhizobium* RCM6 (R1) native to dune Massa is endowed with great effectiveness in improving the growth and nutrition of the host plant.

The study also demonstrated the positive role of the tripartite association performed by double inoculation with rhizobia and endomycorrhizal complex from Lamzar dunes. The stress tolerance of the double inoculation is probably related to the roles of endomycorrhizal fungi in increasing the relative water content and the levels of phosphorus and potassium in stressed *A. cyanophylla.* The water deficit is a limiting factor of the vegetation in arid and semi-arid areas and particularly of coastal dunes. However, in these zones, this factor is related to soil salinity. Indeed, the two phenomena are physiologically dependent. The impact of salinity on the behavior of seedlings *A. cyanophylla* in symbiosis was demonstrated in our previous works.

REFERENCES

Aguirreolea J, Sanchez-Diaz M. 1989. CO_2 evolution by nodulated roots in *Medicago sativa* L. under water stress. J Plant Physiol 134: 598-602. DOI: 10.1016/S0176-1617(89)80154-3

Aparicio-Tejo PM, Sanchez-Diaz J, Pena I. 1980. Nitrogen fixation, stomatal response and transpiration in *Medicago sativa, Trifolium repens* and *T. subterraneum* under water stress and recovery. Physiol Plant 48: 1-4. DOI: 10.1111/j.1399-3054.1980.tb03209.x

Aroca R, Porcel R, Ruiz-Lozano JM. 2007. How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? New Phytol 173: 808-816. DOI: 10.1111/j.1469-8137.2006.01961.x

Augé RM. 2001. Water relations, drought, and VA mycorrhizal symbiosis. Mycorrhiza 11: 3-42. DOI: 10.1007/s005720100097

Barea JM, Werner D, Azcón-Guilar C, Azcón R. 2005. Interactions of arbuscular mycorrhiza and nitrogen-fixing symbiosis in sustainable agriculture. In: Werner D, Newton WE (ed) Nitrogen fixation in agriculture, forestry, ecology and the environment. Springer, Dordrecht.

Bárzana G, Aroca R, Paz JA, Chaumont F, Martinez-Ballest MJ, Carvajal M, Ruiz-Lozano JM. 2012. Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Ann Bot 109: 1009-1017. DOI: 10.1093/aob/mcs007

Blanchet R, Gelfi N, Bosc M. 1977. Relation entre consommation d'eau et production de divers types variétaux de Soja (*Glycine max*. L. Merr.). Ann Agro 28: 261-275.

Bouizgarne B, Oufdou K, Ouhdouch Y. 2015. Actinorhizal and Rhizobial-Legume symbioses for Alleviation of abiotic stresses. In: Arora NK (ed), Plant Microbes Symbiosis: Applied Facets, Springer, India.

Evelin H, Giri B, Kapoor R. 2012. Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. Mycorrhiza 22: 203-217. DOI: 10.1007/s00572-011-0392-0

Fagbola O, Osonubi K, Mulongoy SA, Odunfa S. 2001. Effects of drought stress and arbuscular mycorrhiza on growth of *Gliricidia Sepium* (Jacg.), Walp. and *Leucaena leucocephala* (Lam) de wit. In simulated eroded soil conditions. Mycorrhiza 11: 215-223. DOI: 10.1007/s005720100114

Gong M, Tang M, Chen H, Zhang Q, Feng X. 2013. Effects of two *Glomus* species on the growth and physiological performance of Sophora davidii seedlings under water stress. New Forests 44: 399-408. DOI: 10.1007/s11056-012-9349-1

Hernandez-Sebastia C, Piche Y, Desjardins Y. 1999. Water relations of whole strawberry plantlets in vitro inoculated with *Glomus intraradices* in a tripartite culture system. Plant Sci 143: 81-91. DOI: 10.1016/S0168-9452(99)00014-X

Hatimi A. 1995. Symbiotes racinaires de trois legumineuses arborescentes de dunes littorales de Souss-Massa. Ed. INRA, Paris, Les Colloques 77: 183-190.

Hatimi A, Tahrouch S. 2007. Caractérisations chimique, botanique et microbiologique du sol des dunes littorales du Souss-Massa. Biomatec Echo 2: 85-97.

Hatimi A. 1999. Effect of salinity on the association between root symbionts and *Acacia cyanophylla* Lind.: growth and nutrition. Plant Soil 216: 93-101. DOI: 10.1023/A:1004745707277

Huang CY, Boyer JS, Vanderhoef LN. 1975. Limitation of acetylene reduction (nitrogen fixation) by photosynthesis in soybean having low water potentials. Plant Physiol 56: 228-232. DOI: 10.1104/pp.56.2.228

Lal B, Khanna S. 1993. Renodulation and nitrogen-fixing potential of *Acacia nilotica* inoculated with *Rhizobium* isolates. Can J Microbiol 39: 87-91. DOI: 10.1139/m93-012

Marcar NE, Dart P, Sweeney C. 1991. Effect of root-zone salinity on growth and chemical composition of *Acacia ampliceps* B. R. Maslin, *A. auriculiformis* A. Cunn. ex Benth. and *A. mangium* Willd. at two nitrogen levels. New Phytol 119: 567-573.

Marques MS, Pagano M, Scotti MRMML. 2001. Dual inoculation of a woody legume (*Centrolobium tomentosum*) with rhizobia and mycorrhizal fungi in south-eastern Brazil. Agrofor Syst 52 107-117. DOI: 10.1023/A:1010637401475

Marulanda A, Porcel R, Barea J. M, Azcón R. 2007. Drought tolerance and antioxidant activities in *lavender* plants colonized by native drought tolerant or drought sensitive *Glomus* species. Microb Ecol 54: 543-552. DOI: 10.1007/s00248-007-9237-y

Meddich A. 1997. Ecophysiologie des mycorhizes à vésicules et arbusculs des zones arides: effet sur la croissance, la nutrition minérale et la tolérance du trefle (*Trifolium alexandrinum*) au stress hydrique. Thèse de 3ème cycle.

Meddich A, Oihabi A, , Abbas Y, Bizid E. 2000. Mycorhizes à arbuscules des zones arides: biodiversité et rôles dans la tolérance du trèfle (*Trifolium alexandrinum*) au stress salin. Agronomie 20: 1-13.

Mnasri B, Aouani ME, Mhamdi R. 2007. Nodulation and growth of common bean (*Phaseolus vulgaris*) under water deficiency. Soil Biol Biochem 39: 1744-1750. DOI: 10.1016/j.soilbio.2007.01.030

Naya L, Ladrera R, Ramos J, Gonzalez EM, Arrese-Igor C, Minchin FR, Bacana M. 2007. The response of carbon metabolism and antioxidant defenses of alfalfa to drought stress and ro the subsequent recovery of plants. Plant Physiol 113: 259-267. DOI: 10.1104/pp.107.099648

Oihabi A, Meddich A. 1996. Effet des mycorhizes à arbuscules sur la croissance et la composition minérale du trèfle. Cahiers Agricultures 5: 382-388.

Pena JI, Sanchez-Diaz M, Aguirreolea J, Becana M. 1988. Increased stress tolerance of nodule activity in the *Medicago-Rhizobium-Glomus* symbiosis under drought. J Plant Physiol 133: 79-83. DOI: 10.1016/S0176-1617(88)80088-9

Plenchette C, Perrin R, Duvert P. 1989. The concept of soil infectivity and method for its determination as applied to endomycorrhizas. Can J Bot 67: 112-115. DOI: 10.1139/b89-016

Porcel R, Ruiz-Lozano JM. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55: 1743-1750. DOI: 10.1093/jxb/erh188

Ramos MLG, Gordon AJ, Minchin FR, Sprent JI, Parsons R. 1999. Effect of water stress on nodule physiology and biochemistry of a drought tolerant cultivar of common bean (*Phaseolus vulgaris* L.). Ann Bot 83: 57-63. DOI: 10.1006/anbo.1998.0792

Rodier KR. 1984. L'anlyse de l'eau, eaux naturelles, eaux résiduaires, eau de mer. *7ème édition*, Dunod.

Romdhane SB, Aouani ME, Trabelsi M, de Lajudie P, Mhamdi R. 2008. Selection of high nitrogen-fixing rhizobia nodulating chickpea (*Cicer arietinum*) for semi-arid Tunisia. J Agron Crop Sci. 194: 413-420. DOI: 10.1111/j.1439-037X.2008.00328.x

Romdhane SB, Trabelsi M, Aouani ME, de Lajudie P, Mhamdi R. 2009. The diversity of rhizobia nodulating chickpea (*Cicer arietinum*) under water deficiency as a source of more efficient inoculants. Soil Biol Biochem 41: 2568-2572. DOI: 10.1016/j.soilbio.2009.09.020

Ruiz-Lozano JM, Azcon R. 1997. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95: 472-478

Ruiz-Lozano JM, Collados C, Barea JM, Azcón R. 2001. Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytol 151: 493-502. DOI: 10.1046/j.0028-646x.2001.00196.x

Ruiz-Lozano JM, Porcel R, Aroca R. 2006. Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes? New Phytol 171: 693-698. DOI: 10.1111/j.1469-8137.2006.01841.x

Sanchez-Diaz M, Aguirreolea J, Goicochea MC, Antolin MC. 1995. Limitations de la fixation symbiotique d'azote et autres aspects physiologiques des légumineuses des zones méditerranéennes. Ed. INRA, Paris, Les Colloques 77: 11-29.

Sanchez-Diaz M, Pardo M, Antolin M, Pena J, Aguirreolea J. 1990. Effect of water stress on photosynthetic activity in *Medicago-Rhizobium-Glomus* symbiosis. Plant Sci 71: 215-221. DOI: 10.1016/0168-9452(90)90011-C

Serraj, R, Sinclair TR, Purcell LC. 1999. Symbiotic N_2 fixation response to drought. J Exp Bot 50: 143-155.

Sheehy JE, Minchin FR, Witty JF. 1985. Control of nitrogen-fixation in a legume nodule: An analysis of the role of oxygen diffusion in relation to nodule structure. Ann Bot 55: 549-562. DOI: 10.1093/oxfordjournals.aob.a086930

Shoushtasi NH, Pepper IL. 1985a. Mesquite rhizobia isolated from the Sonoran desert: Physiology and effectiveness. Soil Biol Biochem 17: 797-802. DOI: 10.1016/0038-0717(85)90135-X

Shoushtasi NH, Pepper L. 1985b. Mesquite rhizobia isolated from the Sonoran desert: Competitiveness and survival in soil. Soil Biol. Biochem 17: 803-806. DOI: 10.1016/0038-0717(85)90136-1

Sinclair TR, Purcell LC, Vadez V, Serraj R. 2001. Selection of soybean (*Glycine max*) lines for increased tolerance of N2 fixation to drying soil. Agronomie 21: 653-657.

Soria RD, Correa NS, Rosas SB. 1996. Effect of water stress on the Guar-*Bradyrhizobium* system using PEG 6000. Phyton-Intl J Exp Bot 58: 97-106.

Sprent JI, EK. James. 2007. Legume evolution: where do nodules and mycorrhizas fit in? Plant Physiol 144: 575-581. DOI: 10.1104/pp.107.096156

Sprent JI. 1976. Water deficits and nitrogen-fixing root nodules. *In:* Kozlowski TT (ed). Water deficits and plant growth. Academic Press, New York.

Sprent JI. 1981. Nitrogen fixation. In: Paleg LG, Aspinall D (eds): The Physiology and Biochemistry of drought resistance in plants. Academic Press, Sydney.

Trouvelot A, Kouch J, Gianinazzi-Pearson V. 1986. Mesure du taux de mycorhization VA d'un système radiculaire: Recherche de méthode d'estimation ayant une signification fonctionnelle. Les mycorhizes: Physiologie et Génétique. 1er Séminaire, Dijon, Ed. INRA, Paris.

Wu QS, Xia RX, Zou YN. 2008. Improved soil structure and *citrus* growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. Eur J Soil Biol 44: 122-128. DOI: 10.1016/j.ejsobi.2007.10.001

Wu QS, Zou YN,. Xia RX, Wang MY. 2007. Five *Glomus* species affect water relations of *Citrus tangerine* during drought stress. Bot Stud 48: 147-154.

Zou N, Dart PJ, Marcar N. E. 1995. Interaction of salinity and Rhizobial strain on growth and N2-fixation by *Acacia ampliceps*. Soil Biol Biochem 27: 409-413. DOI: 10.1016/0038-0717(95)98611-Q

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Work environment and the performance of forest rangers in South West Mau Forest, Kenya

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Abstract. *Etemesi NI, Sirmah PK, Chepkwony J. 2018. Work environment and the performance of forest rangers in South West Mau Forest, Kenya. Asian J For 2: 46-53.* Forest protection requires strong governance in the implementation, compliance and enforcement of laws and policies. This can only be achieved if personnel responsible for such duties are equipped with good working environment. The objective of this research was to evaluate and understand how the working environment of forest rangers under Kenya Forest Service (KFS) affects their performance. The Rangers, under the Enforcement and Compliance Division (ENCOM) of KFS, are mandated to implement the enforcement of laws and policies pertaining to forests and their allied resources as prescribed in the Kenya Forest Act of 2005. Qualitative research approach was employed in the data collection by the use of structured questionnaires in four forest stations, namely three stations in Londiani, Masaita, and Sorget of Kericho Zone, Mau complex, and one station in the Kericho Ecosystem Conservator's Office. From a total of 46 rangers deployed in the four stations, 32 rangers were sampled randomly, 8 serving in each of forest stations. A pre-tested questionnaire on demographic trends, duration of service, work environment variables constituting remuneration, living conditions, motivation, appraisals, rewards, empowerment, communication, work tools, mobility, uniforms, challenges, and personal life were administered in January 2016. The performance indicators gave dissatisfaction rates of 59% and 63% in most of the parameters tested. Comparison of the finding of this study with the findings of the surveys of 2010 and 2013 in different conservancies in Kenya gave an index of 51.4% and 56.74% satisfaction respectively. The results, therefore, denote a progressive correlation between the working conditions drivers and the performance of forest rangers. More radical measures must be undertaken to enhance the performance and productivity of rangers through motivation, improving both their intrinsic and extrinsic working environment.

Keywords: ENCOM division, forest rangers, working conditions, working performance

INTRODUCTION

Escalated climate change and degradation of natural resources in the last two decades have encouraged societies across the world to put more attention on governance as well as the implementation, compliance and enforcement of natural resource policies and laws. In the context of climate change mitigation, Warchol and Kapla (2012) note that forestry is one of the key sectors where policies and laws should be enforced. Ultimately, sustainable forest management demands a balance among conservation, regeneration, management, governance, enforcement, and implementation programs (Koontz 2007).

Kenya holds high level of diversity in flora and fauna in its forests, which stretch from the mangroves in the coastal regions to tropical and dry forests (Gichora and Bargerei 2015). Forests offer a myriad of services and products such as eco-tourism, water catchment towers, habitat for wildlife, provision to timber, firewood, and other non-forest products which significantly contributes to the improvement of the livelihood of the forest adjacent communities and a key contributor to the national GDP (Onyango 2013).

Kenya like other parts of the world faces an alarming rate of forest destruction and degradation; over 12,000 hectares of forests are lost yearly due to conversion of forest land to agriculture and other development projects (Kenya Forest Service 2010). Subsequently, considerable extents of forests have continually degraded due to illegal logging, unsustainable utilization, uncontrolled grazing, pole making, and charcoal making (Envag Associates 2013; Onyango 2013; Mathu 2007). Demand for energy is a critical proponent for the exacerbated destruction of forest as more than 70% of national energy is derived from fuelwood and charcoal that is often illegally extracted from forests (Kenya Forest Service 2010). As per the July-September 2014 Kenya Forest Service (KFS) report, Kenya’s forest cover was estimated 6.99% (indigenous forests, plantations, woodlands, and mangroves) which dropped from the initial 12% cover as a result of unsustainable and illegal exploitation of the resource.

In order to curb the exacerbating rates of destruction of forest resources, more radical measures need to be adopted (Mathu 2007; Kimani 2012; Onyango 2013). Under the Kenya Vision 2030 goals, KFS aims to attain a minimum of 10% forest cover (Kenya Forest Service 2010). Establishment of forest enforcement and compliance division is therefore an inevitable option so that the forest resources in Kenya can be protected from degradation (Kimani 2012). Under the Forest Department, the mandate of forest protection as well as law compliance and enforcement is bestowed to the forest guards currently referred to as the Forest Rangers under the Forest Act 2005 (Kenya Forest Act 2007; Kenya Forest Service 2012).

In Kenya, the forest rangers fall under the Enforcement and Compliance (ENCOM) Division established in February 2007 through the Forest Act 2005, which reformed the Forest Department to Kenya Forest Service (Kimani 2012). Under Part V of the Act, the commissioned officers (forest rangers) are mandated to implement and enforce policies and laws pertaining to forests and their allied resources with reference to the prescriptions in the Forest Act (Kenya Forest Act 2007) which was amended to Forest Conservation and Management Act 2016 in September 2016. In addition to the compliance and enforcement of forestry regulations, the officers also provide guidance and supervision in forest management such as forest harvesting, planting, and firefighting; intelligence, inspection, and prosecution of forest offenders; as well as provision of skills and manpower where needed (Kenya Forest Act 2007; Kenya Forest Service 2010). These responsibilities match those of forest rangers and conservation officers in many regions of the world, such as South Africa and United States of America, where the officers take an active role in the law enforcement through crime investigation, arrest of offenders, preparation of cases for court and testifying at trial among others (Warchol and Kapla 2012; Koontz 2007). The ENCOM Division has more than 2500 officers posted in the 210 forest stations and other administrative posts (Envag Associates 2013). They work hand-in-hand with the professional and technical cadre of the Service, forest adjacent communities and other environmental agencies and organizations in their areas of jurisdiction (Kenya Forest Service 2010; Envag Associates 2013). The expected responsibilities are a clear ground for the ENCOM division to upscale its performance; a deliberation that KFS itself must ensure the rangers is capacitated with an ambient and amiable environment from which they can laconically partake their enforcement and compliance duties.

Forest rangers in Kenya and globally play an imperative role in the protection, management, and conservation of forest resources and biodiversity (Eliason 2006; Mathu 2007; Kimani 2012; Warchol and Kapla 2012; New York State Department of Environmental Conservation 2012). The state and condition of forests greatly depend on the ability of the protection agencies to implement their duties amicably and effectively. Mathu (2007) argues, no matter how good policy or law may be; the policy is prone to failure if the enforcement, compliance and implementation measures of the policy are substandard and equivocal. Mathu (2007) and Koontz (2007) further state that enforcement and compliance division in forestry department experiences considerable challenges that potentially inhibit the policy and law enforcement. Some of the noted challenges constantly being faced by forest rangers include poor working conditions, feeble and inadequate structural capacities, inadequate working facilities and equipment, and low morale and motivation (Mathu 2007; Koontz 2007; Kimani 2012; Onyango 2013). These drivers are greatly manifested in the dire calls for promotion of protection, management, and conservation of forests and allied resources (Onyango 2013). Such problems need to be solved by improving the working condition of the rangers (Drizin 2003; Harter et al. 2004).

Working environment affects the productivity, effectiveness and efficiency of any workforce (Oswald 2012; Anderson 2013; Harter et al. 2004). In order to improve the performance of the workforce, it is essential to understand and acknowledge the factors influencing its productivity and efficacy (Harter et al. 2004, Oswald 2012). Kehoe and Wright (2010) note that the working conditions directly affect the morale of the employees; therefore, poor working conditions significantly impede the productivity of any workforce. It is of no exception that the rate of dissatisfaction index recorded in this study inhibits the Rangers' performance. The achievement of the Kenya Forest Service (KSF) objectives and vision rests on the collective ability of all the divisions of the Service and other stakeholders to undertake efficiently their duties (Koontz 2007; Kimani 2012).

This study aimed at evaluating the magnitude at which the working condition implicates the performance of forest rangers. Using the data derived from the questionnaires, the study, therefore, investigated how remuneration rates and motivation, living conditions, transport facilities, and working equipment, workplace discrimination and work-personal life balance of the rangers implicate their work productivity.

MATERIALS AND METHODS

Study area

This study was carried out in Londiani, Sorget, and Masaita forest stations, and the Kericho Ecosystem Conservators base in Kericho Zone in Mau complex, Kenya. The four forest stations are under the protection of 46 forest rangers and 2 inspectors, 1 located at the Kericho Ecosystem Conservators office in located Masaita Forest Station and the other at Londiani Forest College based in Masaita Forest Station. Kericho Zone is of strategic importance to Kenya Forest Service since it houses the only forest rangers paramilitary training situated at Londiani Forest College, in the Masaita Forest station. This was critical for the study being the base from which all the forest rangers in the country are trained; hence, it was anticipated that the concerns and needs of the rangers within the location would be much less.

Sampling

The methodology used was based on the qualitative research approach by the use of questionnaires and analyzed using descriptive statistics. Pre-tested questionnaires were administered to 32 out of the 46 forest rangers serving in Sorget, Londiani, Masaita forest stations, and Kericho Ecosystem Conservator station. According to Krejcie and Morgan (1970) as designed in Morgan's table of determining sample size, out of the 40 targeted forest rangers, only 32 accepted to take part in the research, hence a deviation of 8 as per Morgan's table sample size.

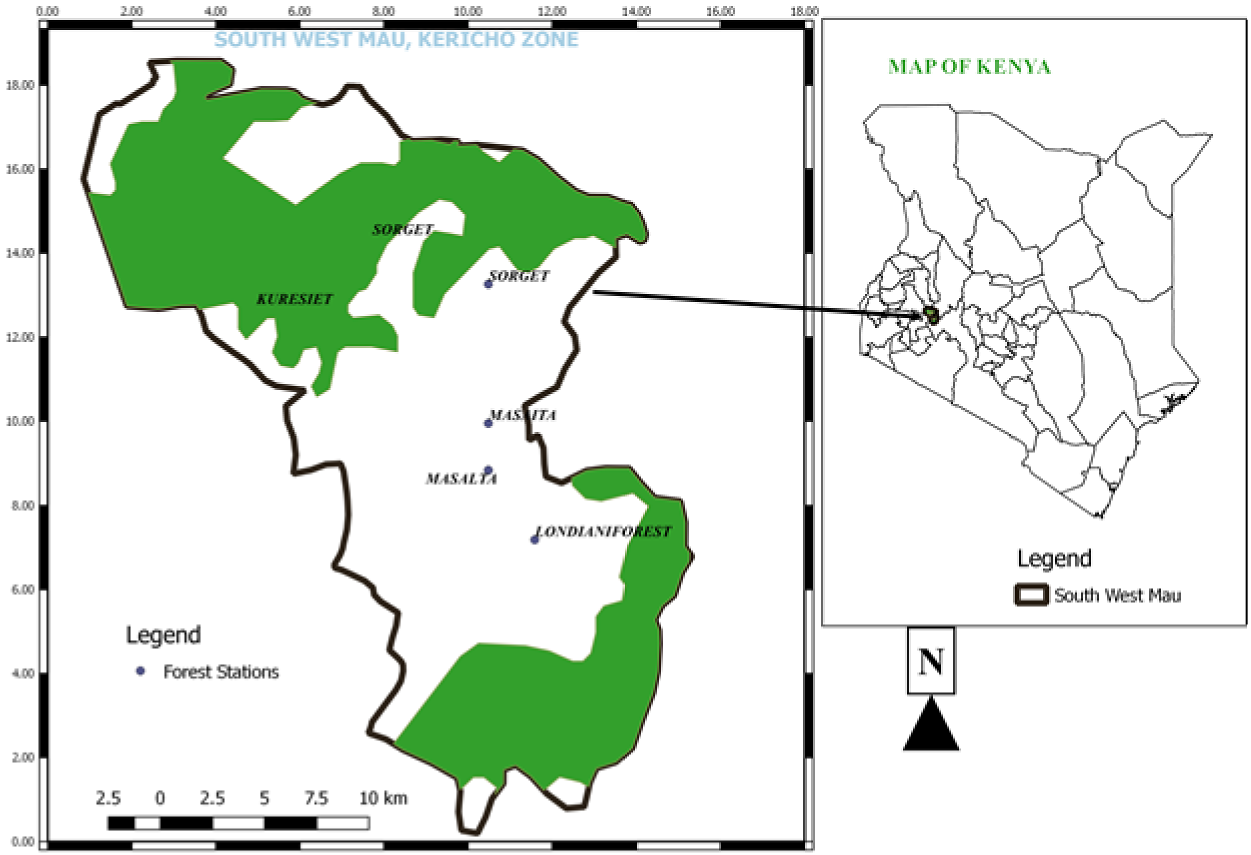

Figure 1. A map of the sampled stations in South West Mau, Kericho Zone, Kenya

Questionnaire administration

The pretested self-administering questionnaires were distributed to the 32 forest rangers who took part in the study. The questionnaires were designed to assess (i) the demographic characteristics {gender, age range, marital status, and work experience} of the workforce and (ii) the drivers of work productivity. The parameters of work productivity examined consisted of the (i) remuneration rates {salaries, job appraisal and promotion}; (ii) workplace motivation {work motivation, communication, and work performance, stress freeness of the working environment and supervisor motivation}; (iii) sensitivity of KFS to rangers needs and wants {wants, needs, and treammwork}; (iv) quality of working facilities and equity {transport, working equipment, uniforms, living conditions, and discrimination} and (v) work-personal life balance {balance and freeness with supervisors}. The questionnaires were collected on the third day after being issued to the respondents.

Data analysis

The questionnaires were cross-examined to ascertain their accuracy, uniformity, and completeness. It was necessary to ascertain the responses before organization and coding to remove incomplete questionnaires to increase the accuracy. The questionnaires were then organized, numbered, and coded using the Statistical Package for Social Scientists (SPSS) version 22. The data was then analyzed using descriptive statistics and represented in frequencies and percentages using charts and tables to validate the relationships between the different variables. The results were analyzed and interpreted in frequency distribution tables.

RESULTS AND DISCUSSIONS

Demographic trends of forest rangers

The demographic data of the forest rangers were assessed to know the work diversity of the officers as the diversity in any workforce is attributed to sustainable and improved performance through sharing of experiences and skills. Gender is one of the prime indicators of work diversity of which the Kenya Constitution (2010) under Article 27 (8) categorically states, "*The State shall take legislative and other measures to implement the principle that not more than two-thirds of the members of elective or appointive bodies shall be of the same gender*". The law demands that a gender balance of workers within its parastatals as well. Out of the 32 respondents who took part in the study, forest rangers were 75% male and 25% female. It is important to verify the balance in the distribution of gender and age in the workforce; this affirms the relationship in the workforce and adheres to the gender-balance call as per the Kenya Constitution.

Ageism is one of the imperative outliners of work diversity. Thomas et al. (2014) state that age is organizing principle and an embodied identity as well as signifier of the richness and stability of a workforce in contemporary society. They note that age distribution significantly influences the material effect of the organization affecting both the individuals and organizations productivity. Thomas et al. (2014) study recommended for an age balance in any workforce to ensure flexible, energized, dependable, and sustainable transition in the workforce. The age diversity in this study presented skewed and formidable distribution index. Majority of the ranger (over two-thirds) fall under the age of 31 to 44 years old (Table 1).

The distribution imbalance in age might posses a danger in terms of transition of responsibilities and experiences. Envag Associates (2013) attributes the skewness to the long and inconsistent recruitment of forest rangers; it is a challenge and raises alarms for the sustainability of the workforce in the next 15 years if more rangers are recruited. The ENCOM Division is dominated by progressive old age lacking youthfulness in that only 12% of the rangers are aged 18-24 and 25-30. The age of 31-44 is the majority of the forest rangers. Therefore, a balance of the workforce is crucial for sustainable performance of the forest rangers.

The experience of the workplace viably denotes its diversity and stability (Anderson 2013). Just like age, the experience of the employees promotes organizational sustainability, re-energizing the workforce through the integration of skills and experiences of the young and older employees. The research asked the respondents regarding the duration they have worked with ENCOM Division. It is noted about half of the rangers have worked with the division for at least 10 and 20 years. The experience is relatively balanced, unlike the age.

Table 1. Distribution of age range of the respondents

Age range	%
18-24	12
25-30	12
31-44	72
50 and above	4

Table 2. Distribution of working experience of the respondents

Forest rangers duration of service (years)	%
1-5	22
6-10	26
11-15	37
16-20	8
21-25	4
26-30	3

Impacts of remuneration rates on the working performance

Remuneration rates and job appraisals

According to a report on strengthening of the forest law enforcement and governance by the World Bank (2006), it is of critical importance for the initiation of institutional reforms of forest law enforcement agencies. The report conjectures on the vitality of the forest management agencies to acknowledge and understand the role of incentives such as the salary structures and job appraisal among others on the productivity of its officers. Many studies have been conducted to evaluate the magnitude at which remuneration affects the performance of employees; many have diverse findings (Van Herpen et al. 2005; Oswald 2012; Warchol and Kapla 2012; Kimani 2012; World Bank 2006; Onyango 2013).

The findings of the study indicated that the forest rangers were equally dissatisfied with the current remuneration rates, job appraisal and promotion measures implemented by the Service. On both the remuneration and job appraisal questions, the rangers were 59% dissatisfied with the salaries and promotion structures (Table 3). This implies that a great magnitude of the ranger performance was influenced by these factors. Van Herpen et al. (2005) argue that even though salaries and job promotions do not directly depict the working environment, salaries are a crucial element of extrinsic motivation while job appraisal favors intrinsic motivation. Motivation levels are noted to significantly implicate work productivity, as Warchol and Kapla (2012) argue in their study that low salaries often contribute to escalated dissatisfaction rates and low motivation among South African law enforcement and conservation officers. The low motivation is likely to induce them to the vices of corruption and bribery, low patrol frequencies, feeding offenders with critical information about the richness of the forests and where to find high-quality fauna and flora (Warchol and Kapla 2012). Consequently, Kimani (2012) explained that even though it is common parlance and belief that meager salaries promote the low motivation manifested by corruption, salaries and job appraisal alone are not sufficient to spur the performance of the rangers, rather the integrated improvement of both the motivation factors and the working conditions. Onyango (2013) equally notes that high and long-term performance of employers is promoted by stable remunerations, promotion measures, and work environment; these facts are determinants for better productivity.

Table 3. Level of satisfaction of rangers on remuneration rates, and job appraisal and promotions incentives.

Rangers response	Salaries (%)	Job appraisals and promotion %
Strongly Disagree	4	0
Disagree	55	59
Strongly Agree	11	18
Agree	26	19
Don't know	4	4

Job appraisal is projected towards promoting the intrinsic needs of the workforce, motivating them to upgrade and transcend their duties (Van Herpen et al. 2005). An employer that recognizes the efforts of employees motivates them to upscale their efforts, which manifests in cumulative productivity. In the 2010/2011 KFS annual report, 41 vehicles were impounded by rangers; 262 forest offenders were prosecuted; 197 structures illegal erected in forest land were demolished; over 48 tons of timber of various species, 9817 frames of cedar posts, doors and door frames, 12 power saws, and 12 drawn carts of donkeys were impounded while 498 bags of charcoal were recorded (Kenya Forest Service 2010). Based on these statistics in relation to the recorded rates of displeasure, if more measures to promote the motivation of the rangers are undertaken, immense and improved performance shall be attested.

Work motivation and work performance

Moldogaziev and Fernandez (2011) perceive a view that the performance of employees is strongly attributed to the magnitude at which the workforce is empowered and motivated. Besides salaries and job appraisals, KFS through the ENCOM Division structures and programs can undertake various incentives that would promote the motivation of the rangers. Drawing insights from this view, the study assessed the levels at which the rangers were satisfied with the motivation measures deployed by KFS, their supervisors, the communication abilities of the unit and the stress freeness of their working environment. Table 4 outlines the feedback of the respondents.

Out of the 32 respondents, 47% equally recorded to be demotivated and satisfied with the work motivation measures undertaken by KFS such as outdoor activities and competitions. This is an indication the service is averagely motivating the rangers through their activities. The respondents also noted 59% disregard rate of the motivation programs undertaken by their supervisors. This implies no matter what the efforts the general ENCOM units undertake if it is not applied equivalently to all forest stations and equally to all the rangers, the Division is prone to ever record ubiquitous performance. The motivation should be equitably stemming from both the professional cadre (forest managers) and the ENCOM commander, inspectors, and corporals. KFS is based on a vertical organizational structure, the equity in the motivation status is likely to enhance the performance of the rangers across all forest stations across the country (Envag Associates 2013). If the supervisors of forest station and the Rangers fail in implementing their duties, it is inevitable for the forest stations to experience ever-high rates of destruction of forest resources.

In addition to poor work and supervisor motivation, the working environment of the rangers was overwhelmingly considered as unconducive. Sixty-three percent of the respondents registered discontent with their working environment noting it to be stressful due to lack of proper working gear among others. Oswald (2012) study on the implication of the working environment on the performance of health care workers in Tanzania noted that poor and stressful working conditions as perceived by the employees are a coherent ingredient of poor performance. Oswald argues that creation of a supportive environment has the potency of enhancing the efficacy of the workers. It is of paramount importance for the Service to determine and mitigate the stressful elements perceived by the rangers to enable them to improve their forest enforcement and compliance responsibility scorecard. It is argued by Moldogaziev and Fernandez (2011) that the amiability of the working condition amicably correlates with job satisfaction and productivity. This study elucidated a very high rate of stress factors in the environment, hence calling for radical measures to improve the working conditions of the rangers.

On the other hand, the rangers acknowledged the communication capacities of the rangers to be efficient. Seventy-four of the respondents approved the communication channels and programs provided. If the Service can provide efficient communication, it has the potential for extending the same to other sectors. Kimani (2012) and Mathu (2007) point out that due to technological advancements, it is also prudent for the Forest Services and departments to adopt new communication technologies that can further improve the working efficacy of the rangers.

Sensitiveness to Rangers' needs and concerns

In every working environment, there are specific needs anticipated by the workforce that they value and greatly perceived to motivate their performance when met. For instance, Warchol and Kapla (2012) note that it is a need for the conservation and forest rangers to be in a group of at least three and with sufficient gears when taking patrol duties. The needs and concerns varied extensively from forest station to station which many stem from decency of housing to prosecution of offenders. With this view, the researchers assessed the degree to which KFS promotes teamwork and its sensitivity to the needs and concerns of the forest rangers.

Table 4. Extent at which forest rangers supervisors motivate them to improve their performance by providing timely and constructive communication

Rangers response	Work motivation %	Supervisor motivation %	Communication and work performance %	Stress-free %
Strongly disagree	4	3	0	8
Disagree	55	44	26	55
Strongly agree	11	15	63	14
Agree	26	32	11	22
Don't know	4	6	0	0

Table 5. Sensitivity of KFS to forest rangers' concerns and needs and promotion of teamwork

Rangers response	Needs and wants %	Teamwork %
Disagree	63	26
Strongly Disagree	0	4
Strongly Agree	7	26
Agree	30	37
Don't know	0	7

Sixty-three percent of the respondents indicated that Kenya Forest Service is not sufficiently sensitive to the needs and wants of the rangers. Only 37% approved the efforts undertaken by the Service in meeting their requirements and concerns. In a study conducted in 2013, one of the respondents was noted to look forward to a time when all the KFS employees shall be treated equally. Three years later, the rangers were still expressing skewness in the manner in which the Service responds to their needs (Kenya Forest Service 2010). Harter et al. (2004) argue that when the needs of employees are met, they become more involved and enthusiastic, hence promoting work productivity. They as well note the aggravated enthusiasm among employees who consider their employers to be assertive to their concerns creates and promotes a sense of belonging, value, and growth. Employee satisfaction is an antecedent of employee engagement. It is worth noting that employees with a higher job gratification are susceptible to sustainable performance in the long run (Koontz 2007; Eliason 2006).

The ENCOM Division fairly supports and promotes teamwork among its officers; 63% of the respondents indicated there are motivated and encouraged to embrace teamwork while 30% held a contrary view. Collaboration among forest rangers is critical to their success. The rangers patrol and protect large tracks of forests, which require very extensive and coordinated collaboration amongst themselves and other stakeholders, hence fundamental to the protection programs (Onyango 2013; Kimani 2012; Eliason 2006; Oswald 2012). Kozlowski and Bell (2012) noted that encouraging teamwork stimulates the involvement of employees due to paradigm shift from individuality to collectivity, hence mutual commitment and accountability.

Adequacy and quality of transport facilities and work equipment

The study aimed at assessing the magnitude at which the transport facilities and working equipment influenced the performance of the rangers. It was undeniable that about two-thirds of the rangers perceived the transport and work equipment as inefficient and unsustainable.

The above findings are startling as the duties of the rangers revolve around patrol, an activity that requires coherent and efficient means of mobility and equipment such as sufficient firearms and ammunition to counter forest offenders who may have more sophisticated weapons. Sixty-five and sixty-six percent of the respondents discredited the sufficiency and quality of the means of transport and working equipment respectively. Insufficiency of vehicles precisely designed for patrol in the forest terrains makes it impossible for the Rangers to transport even the apprehended culprits and impounded property to police station for booking.

The findings affirm the results in the 2010 and 2013 reports on impacts on working environment on KFS employees (Envag Associates 2013). Poor means of mobility and work equipment increase the workload in the division, in that they can patrol small areas within their reach, leaving other areas vulnerable to exploitation (Warchol and Kapla 2012; Onyango 2013). Transport means are also critical as an emergency response strategy as the rangers as expected to be standing by at any time due to emergencies such as fire and intelligence on imminent and ongoing illegal activities (Onyango 2013). The 2013 study results also indicated that ENCOM staff has a satisfaction index of 48.89% and 41.67% on the adequacy of working tools (firearms, tents, GPS, and other surveillance equipment) and communication equipment (walkie-talkies) respectively (Envag Associates 2013). Lack of these facilities and equipment is a dire impediment to the operations assumed by the staff.

The activities of forest rangers majorly include forest patrols, which predominantly move through the forests. The primary pillars of their work efficiency are bestowed on their mobility potential as well as the work equipment such as firearms and camouflaging uniforms (Onyango 2013). Kimani (2012) points out that the operational efficacy of the forest rangers is rooted in the aptness of transport and work equipment facilities that directly implicate the Rangers actions as they undertake the prevention, detection, and suppression duties. Through activities such as patrols, prevention of forest crimes is combated as they aim at minimizing the opportunities of engaging in illegal acts, so the Rangers need to be able to swiftly move into the forests without detection by adjacent communities who may warn the offenders. The impounded products also need to be transported to the forest stations/beats and police stations; therefore, the inefficient means of transport make their duties futile and predetermined by offenders (Onyango 2013). The Rangers also need to be moved to certain points in the forest before they set on foot patrols to enhance the area covered in each patrol as opposed to walking from their bases into the forests. Onyango's (2013) study indicated that most of the forest stations in the Embu ecosystem in Kenya lacked vehicles in good conditions, hence impeding even the transportation of the arrested offenders to the police stations. The same was noted in this study, only the Masaita forest station and the Kericho Ecosystem base owned vehicles, while the other two depended on the Ecosystem's Conservators office to avail them with vehicles when needed. Consequently, poor working equipment such as raincoats, gumboots, and tents among others renders the work of the ranger's absolute cumbersome at night and during rainy days. These implications are advantageous to the offenders.

Adequacy and quality of uniforms, living conditions, and equity issues

Uniforms, living conditions, and cases of job discrimination against disciplined forces in Kenya attract a lot of attention (Onyango 2013). In this view, it was imperative to understand and evaluate the forest rangers' satisfaction with the adequacy and quality of their living conditions, uniforms, and equity issues.

The results show that the camouflage/jungle and official uniforms issued to the rangers are appropriate and of high quality. Sixty percent of respondents approved the uniforms while 40% viewed them to be inappropriate. Nevertheless, most registered substantial trepidations on the frequency at which uniforms are issued. Twenty-five point nine percent of the respondents preferred the uniforms to be issued whenever they are worn out, 22.2% after every two years, 14.8% after every five years, while the majority (37%) desired the uniforms to be issued yearly.

The living condition of the forest rangers recorded the highest rate of displeasure; majority of the rangers (78%) termed the living condition to be archaic, inhabitable and deplorable. Most of the houses are made of timber during the colonial era and lacked electricity and water, and neither are they regularly maintained. Kimani (2012) registered displeasure with the archaic status of the houses of the rangers. He argues that provision of better living conditions is an essential work motivation factor that the KFS has overlooked for so long hence beheading the rangers morale. KFS should prioritize improving the housing conditions of the rangers by constructing better and decent housing units as well as renovating the existing permanent structures. The working environment proved equitable and non-discriminating; 85% of the respondents appreciate the efforts of KFS in ensuring they are treated equally and fairly.

Work-personal life balance

The study noted that 97% of the respondents are able to keep a reasonable balance between their work and their personal life; thus, the balance does not limit their performance. In addition to personal life-work balance, 59% of responses showed the ranger is free to discuss their personal life and professional problems with their supervisors without fear of victimization.

Table 6. Adequacy and quality of the transport facilities and working equipment

Rangers response	Transport %	Working equipment %
Strongly disagree	9	11
Disagree	56	55
Strongly agree	13	8
Agree	22	26

Table 7. Responses on the appropriateness and quality of uniforms

Rangers response	Uniforms %	Living %	Discrimination %
Disagree	40	63	11
Strongly disagree	0	15	4
Strongly agree	4	7	30
Agree	56	15	55

Table 8. Personal life - Work environment balance

Rangers response	Balance%	Freeness%
Strongly disagree	0	4
Disagree	3	37
Strongly agree	20	15
Agree	77	44

Conclusion and recommendations

From the data analysis and the discussion above, it is evident that the performance of forest rangers in respect to the findings of this study is deteriorating at an alarming rate. The dissatisfaction rates of 59% and 63% in most of the parameters of the study indicate that their performance is compromised by many factors. Comparison of the finding of this study with the findings of the surveys of 2010 and 2013 depicts a large disparity, the dissatisfaction index of this study ranges between 59-63% compared to 51.4% and 56.74% satisfaction index of 2010 and 2013 surveys respectively (Kenya Forest Service 2010; Envag Associates 2013). The ENCOM Division has a huge responsibility to protect forests in Kenya; hence, a record of such low rates of satisfaction should worry every forest manager, conservationist, and policy-makers since the Rangers play an imperative role in the enhancing sustainability of future forests. Forest illegal activities in natural forests such as charcoal making and illegal logging have been on the rise, an indication of the poor performance of forest rangers because of the impoverished working conditions as noted in this study.

ENCOM Division has direct responsibility and large task to improve the working conditions and environment of the rangers. No matter how efficient conservation and regeneration programs may be undertaken, while enforcement lags behind, the cumulative performance of the entire process shall ever record poor performance. Measures that are more radical must be undertaken to enhance the performance and productivity of rangers by motivating them as well as acknowledging impediments of low employee satisfaction on workforce performance.

Some of the measures KFS can partake to promote the functionality and performance of the ENCOM cadre comprise provision of modern patrol facilities such as air patrol facilities (choppers), vehicles, and motorbikes specifically designed for forest terrain. These will enable rangers to access remote and impermeable areas as well as cover large areas over a short time. Better and adequate communications and surveillance amenities (such as walkie-talkies, hotlines, GPSs and GIS training) must also be facilitated, as they will improve communication and collaboration abilities among the officers and community forest policing activities, hence boosting their abilities to curb ongoing illegal operations. In addition, it is crucial for the division to be liquidated with sufficient and appropriate working tools. The ranger should be provided with a rifle, sufficient ammunition, gumboots due to the terrain and weather of Mau Complex, well-conditioned tents, and camping facilities, as well as frequent issuance of uniforms among other facilities and equipment, deemed necessary.

KFS should also consider revising salaries and the remunerations rates offered in accordance with the national and international stipulated pay grades. If in agreement, it should devise other mechanisms and incentives that would escalate the Rangers' motivation such as job appraisal and promotions. The promotions should be conducted on an equal and fair basis with profound emphasis on the cumulative performance of each ranger, work experience, and levels of education.

The housing conditions have recorded the highest distressing rate of dissatisfaction, KFS should make it a priority. It should, therefore, construct permanent and decent housing units as opposed to timber ones, frequently renovating the existing ones, and at least supply them with electricity and tap water. The Rangers should be regularly taken to seminars and refresher courses on which they can be trained in customer care and polish their performance as a token of motivation.

REFERENCES

Anderson A. 2013. Factors affecting employee performance. Demand Media, Kenya.

Drizin M. 2003. Worker loyalty is pretty low. United Press International: http://www.upi.com/Business_News/2003/09/01/Worker-loyalty-prettylow/UPI-98111062459018/

Eliason SL. 2006. Factors influencing job satisfaction among state conservation officers. Policing Intl J Police Strat Manag 29 (1): 6-18. DOI: 10.1108/13639510610648458

Envag Associates. 2013. Work Environment Survey. A Report Presented To Kenya Forest Service, Nairobi

Gichora M, Bargerei R. 2015. Forestry Society of Kenya Holds Forest Policy Dialogue Meeting. The Forester: A quarterly magazine of the Kenya Forest Service, Nairobi.

Harter JK, Asplund JW, Fleming JH. 2004. Employee engagement and performance: a meta-analytic study of causal direction. Gallup Research Report, Gallup, New York.

Kenya Forest Act. 2007. “Part V – Enforcement,” Kenya Forests Act 2005.

Kenya Forest Service. 2010. In The Forester. A quarterly magazine of the Kenya Forest Service. Kenya Forest Service, Nairobi.

Kenya Forest Service. 2012. Enforcement and Compliance Standard Operating Procedures (Sops) manual. Kenya Forest Service, Nairobi.

Kimani JN. 2012. "The Ranger I have become." The Forester issue No.6.A quarterly magazine of Kenya Forest Service. Kenya Forest Service, Nairobi.

Koontz, T. M. 2007. Federal and state public forest administration in the new millennium. Public Administr Rev 67 (1): 152-164. DOI: 10.1111/j.1540-6210.2006.00704.x

Kozlowski SWJ, Bell BS. 2012. Workgroups and teams in organizations. In: Weiner IB, Schmitt NW, Highhnouse S. (eds.). Handbook of Psychology, Industrial and Organizational Psychology. 2nd ed. John Wiley & Sons, NJ.

Krejcie RV, Morgan DW. 1970. Determining sample size for research activities. Educat Psychol Measur 30 (3): 607-610. DOI: 10.1177/001316447003000308

Mathu W. 2007. Forest Law Enforcement and Governance in Kenya. Kenya Forest Service (KFS), Ministry of Environment and Natural Resources, Nairobi.

New York State Department of Environmental Conservation. 2012. Division of Forest Protection 2012 Annual Report. New York.

Onyango NI. 2013. Factors Influencing Performance of Forest Rangers in Kenya Forest Service: A Case of Embu Ecosystem, Kenya. Kenya Forest Service (KFS), Ministry of Environment and Natural Resources, Nairobi.

Oswald A. 2012. The Effect of Working Environment on Workers Performance: The Case of Reproductive and Child Health Care Providers in Tarime District [Thesis]. Muhimbili University, Health and Allied Sciences, Kenya.

Van Herpen M, Van Praag M, Cools K. 2005. The effects of performance measurement and compensation on motivation: An empirical study. De Economist 153 (3): 303-329. DOI: 10.1007/s10645-005-1990-z

Warchol G, Kapla D. 2012. Policing the wilderness: A descriptive study of wildlife conservation officers in South Africa. Intl J Compar Appl Crim Justice 36 (2): 83-101. DOI: 10.1080/01924036.2012.669911

World Bank. 2018. Strengthening forest law enforcement and governance: addressing a systemic constraint to sustainable development. Institutional and Governance Review (IGR), Report Number 36638. World Bank, Washington, DC.

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Vegetation structure and composition in Ciletuh Geopark, Sukabumi, Indonesia

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Abstract. *Wulandari I, Hendrawan R, Husodo T, Megantara EN. 2018. Vegetation structure and composition in Ciletuh Geopark, Sukabumi, Indonesia. Asian J For 2: 54-61.* Ciletuh Geopark has unique geological and biological features which might provide benefits both to the environment and society. The sustainable management of the geopark requires information on biodiversity elements including the vegetation occurring around Ciletuh Geopark. This research was conducted to determine vegetation communities and plants diversity of the Ciletuh Geopark. The method used was a qualitative method through inventory of plant species and observation of the vegetation profile diagram, which represents a vertical structure of the vegetation community. In general, Ciletuh Geopark had four types of communities, namely natural forests, gardens, agroforest (*talun/kebon tatangkalan*), and beach and mangrove vegetation. In total, there were 179 plant species recorded from understorey to trees including plant species protected by the Indonesian government, namely *Rafflesia patma*. In the geopark, there had been changes in vegetation which is now dominated by crop plant species. This study highlights the importance of conserving the remaining natural vegetation in Ciletuh Geopark to enhance the biological values of the geopark.

Keywords: Ciletuh, geopark, composition, structure, vegetation

INTRODUCTION

In the last 15 years, there is an emerging interest of global community in the establishment and development of geopark. This is a new initiative promoted by UNESCO that aims to conserve areas with high importance not only on geological interests but also regarding biological diversity. So far, larger attention is highlighted on the geological aspects while the biodiversity issues are rather overlooked.

Ciletuh Geopark is located in Sukabumi, West Java, Indonesia. It was established in September 2016 through the Decree of the Governor of West Java No. 556/Kep. 941-Rek/2016. Administratively, Ciletuh Geopark encompasses eight sub-districts, namely Cisolok, Cikakak, Palabuhanratu, Simpenan, Ciemas, Ciracap, Waluran, and Surade. One of the aims of the establishment of this geopark is among others to support sustainable development, especially in Sukabumi and West Java Province.

Particular aspect in Ciletuh Geopark that can be explored to be utilized sustainably is the biodiversity elements. However, since the establishment of Ciletuh Geopark, not many efforts were carried out to reveal information regarding plant and animal diversity in the area. Previous study by Megantara (2016) in Ciletuh Geopark only focused on an inventory of REEPS (Rare, Endangered, Endemic, Protected Species) of animals species. As such, studies need to be expanded to reveal information regarding plants or vegetation of Ciletuh Geopark.

Gem (1996) states that vegetation is a collection of species of plants, each of which is incorporated in a population that lives in habitat and interacts with one another. Interaction in a community is reflected in the structure and composition of vegetation. Stratification in a community occurs because of competition between dominant species with other species or between tall trees in the uppermost layers controlling the trees below (Soerianegara and Indrawan 2005). The interaction between plants gives rise to a characteristic composition of vegetation. Mueller-Dombois and Ellenberg (1974) use the term composition to express the floristic wealth of forests. Soerianegara and Indrawan (2005) add that species composition is distinguished between population (one species) and community (some species). The composition of vegetation is defined as the variation in the plant species that arrange community. The composition of plant species is a floristic list in a community (Misra 1973).

The purpose of this research is to find out plant diversity and vegetation community occurring in Ciletuh Geopark. The results of this study are expected to serve as baseline information for the management of the geopark as well as for future studies looking at the dynamics of the vegetation over a certain period.

MATERIALS AND METHODS

Study area

This study was carried out in Ciletuh Geopark, Sukabumi, West Java, Indonesia. Data collection was concentrated in the horseshoe-shaped buffer area surrounding the Amphitheater (Figure 1). The Amphitheater has high importance since the government plans that this area would be projected as the center of Ciletuh's economic growth. In general, the Amphitheater is located in low elevation so that vegetation occurring in this area can be classified as lowland forest. A previous study by Megantara (2016) showed that the Amphitheater is the habitat animal species with REEPS status.

Methods

Data collection was carried out through an inventory of plant species as well as direct observation to capture the vegetation profiles. Stratification and vegetation profiles were drawn by profile diagram vertically (Mueller-Dombois and Ellenberg 1974). Profile diagram was aimed to understand the structure of vegetation and created based on transect with length of 200 m and plot with size of 10 x 10 m^2. The profile diagram represented vertical structure of vegetation at four types of communities, namely (i) natural forests (Selagedang Hulu, Curug Awang Bawah, Curug Tengah, Puncak Manik, Cipeucang Atas, Curug Dogdog, Curug Cimarinjing), (ii) gardens (Kebun Jati Selagedang, Kebun Kelapa Tamanjaya, Curug Awang Atas), (iii) agroforests (Cigembong, Cipeucang Bawah, Mandrajaya-Ciwaru, Pasir Muncang, Gunung Masigit), and (iv) coastal vegetation/mangroves (Mangrove Cikadal).

The profile diagram depiction was created on millimeter block paper with a scale of 1: 100. In the picture, it is added information such as research title, scale, species code, tree height scale, species name, and location map and study transect.

Data were analyzed using the qualitative-descriptive method to describe the condition of vegetation at the study location. Based on vegetation profile, the canopy strata were formed. The determination of the amount of strata is very dependent on the personal decision of the researcher.

RESULTS AND DISCUSSION

Vegetation community type

There were four vegetation community types found at the study sites in Ciletuh Geopark, namely natural forests, gardens, agroforest (*talun*/*kebon tatangkalan*), and beach and mangrove vegetation. Natural forest and agroforests dominated the landscape in Ciletuh Geopark. The description of each vegetation is as follows.

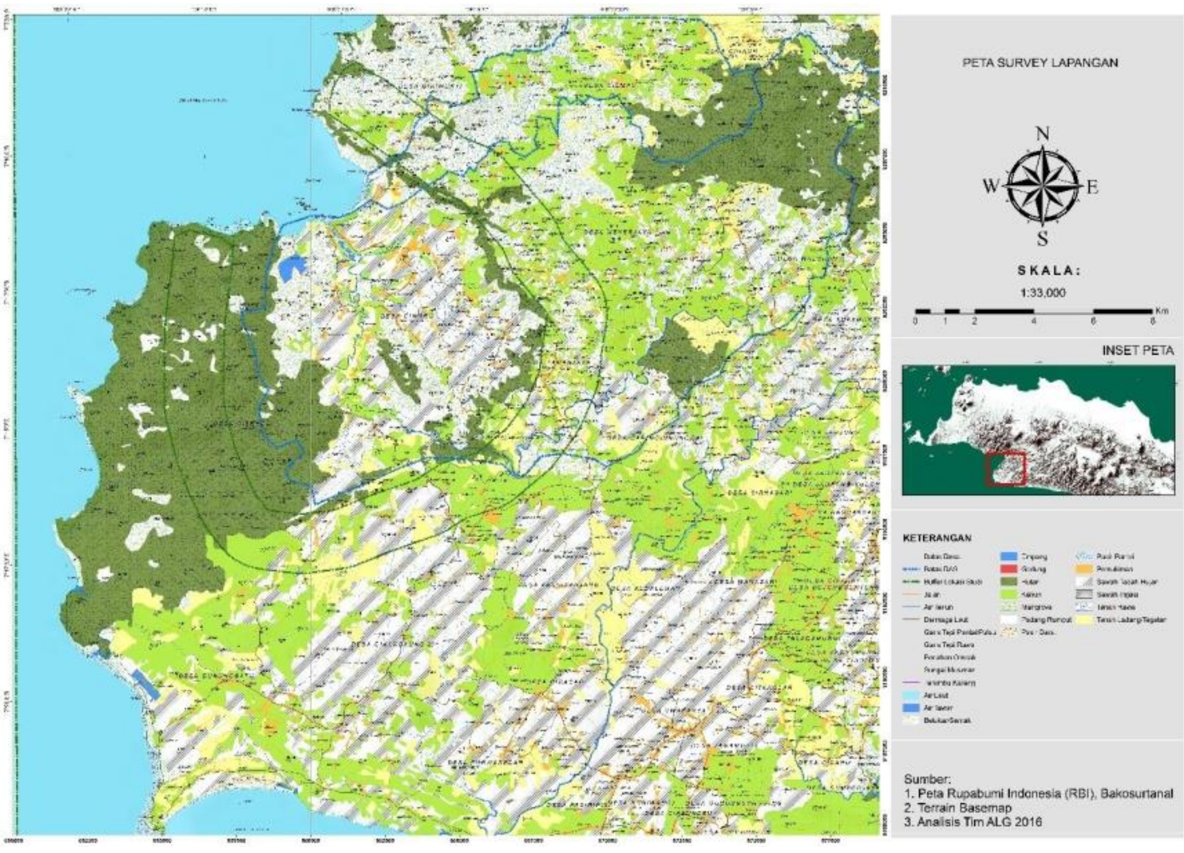

Figure 1. Map of the study location in Ciletuh Geopark, Sukabumi, West Java, Indonesia

Forests

Forest was arranged by natural vegetation. The surrounding area of the forest had undergone land-use change into agricultural land. The forest was located on steep slopes which were difficult to reach, such as around waterfalls and cliffs, thus saving it from encroachment for land clearing. The forest around waterfall had a slope of 50-90%, but the other forest around the Curug Dog-dog had slope between 20-30%, which tends to be flat. Meanwhile, the forest around the cliff has slope between 40-80%. In general, the canopy closure of forests around the cliff tended to be denser while the forest around waterfall was more open although in Curug Dog-dog it had a closed canopy closure (70-80%).

Garden

In general, gardens dominated the landscape in Ciletuh Geoparks. It is arranged by agricultural land consisting of cultivated vegetation, such as horticultural crops. Some gardens were cultivated by single crops (monoculture) while other gardens were planted with various crops to form intercropping vegetation (polyculture). Plant species that were often found in the garden included teak, coconut, rice, corn, and bananas. However, there were species being the main commodities of each garden, such as teak and coconut, which were the main commodities. The gardens had a relatively flat and wavy topography, with a slope of <50%. The canopy closure of the garden was relatively open, so sunlight can penetrate the ground.

Agroforest (Talun/kebon tatangkalan)

In Ciletuh Geopark, agroforest was generally located in a relatively flat area. Canopy closure was not too dense, around 50%. *Talun* which had slope of > 50% and a fairly close canopy closure can be found in Keusik Bodas area with slope of 50-70% and canopy cover of 75-95%.

Beach and mangrove vegetation

Beach and mangrove vegetation communities were located in coastal areas, so plant communities had adapted to high salinity. This area had a broad coastal vegetation formation because most of the land haf been turned into cattle grazing and residential areas. Coastal and mangrove vegetation were separated by village and river roads with relatively flat topography.

Figure 1. Vegetation communities of natural forest in Ciletuh Geopark, West Java, Indonesia

Figure 2. Vegetation communities of garden in Ciletuh Geopark, West Java, Indonesia

Figure 3. Vegetation communities of agroforest (*talun/kebon tatangkalan*) in Ciletuh Geopark, West Java, Indonesia

Figure 4. Vegetation communities of beach and mangrove in Ciletuh Geopark, West Java, Indonesia

Structure of vegetation community

Wyatt-Smith (1963) classify the structure of vegetation communities into four categories, namely trees, poles, saplings, and undergrowth. Tree is woody plants with diameter at breast height (DBH) > 35 cm, pole is a woody plant with a diameter of 10-35 cm, sapling is a woody plant with a diameter of <10 cm or has a height of >1.5 m and undergrowth is vegetation with a height of <1.5 m. Soerianegara and Indrawan (2005) classify vegetation structure based on the level of layers from top to bottom horizontally, namely strata A, B, C, D, and E. Strata A is plants that have a height > 30 m, strata B consists of plants with a height between 20-30 m, strata C consists of plants with a height between 4-20 m, and strata D and E each are plants with a height 1-4 m and < 1 m, respectively.

Generally, the vegetation in the Ciletuh Geopark had complex structures, starting from tree level to the sapling and also arranged by undergrowth vegetation. Based on its stratification, vegetation in the Ciletuh Geopark was dominated by strata B (height between 20-30 m) and C (height between 4-20 m).

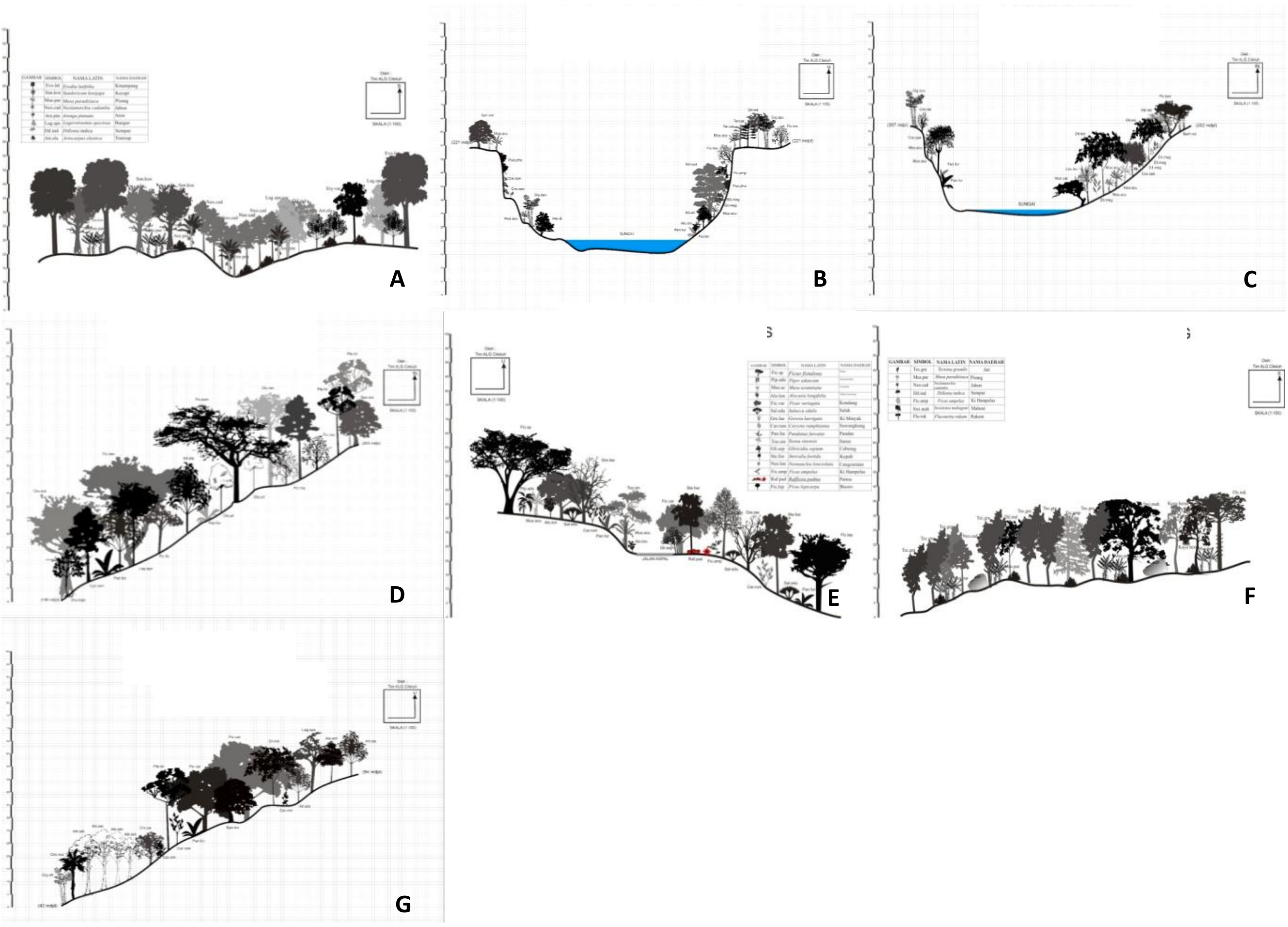

Figure 5. Vegetation profile diagram of natural forest in Ciletuh Geopark, West Java, Indonesia. A. Selagedang Hulu, B. Curug Awang Bawah, C. Curug Tengah, D. Puncak Manik, E. Cipeucang Atas, F. Curug Dogdog, G. Curug Cimarinjing

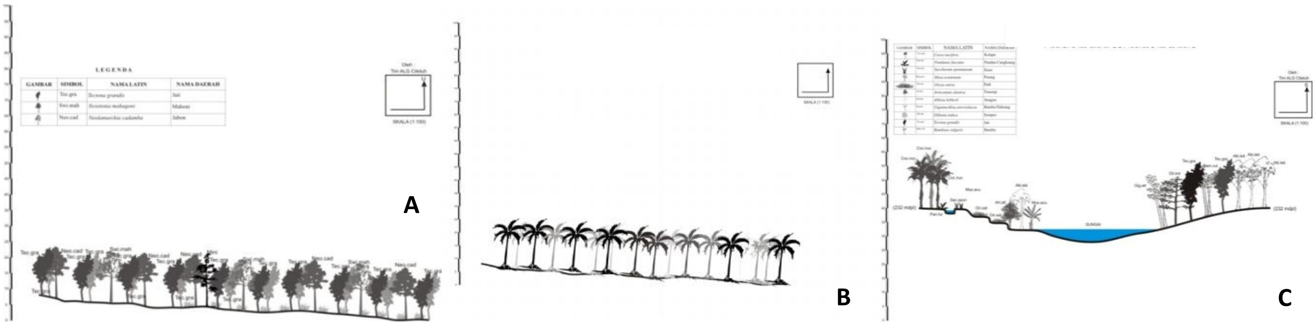

Figure 6. Vegetation profile diagram of garden in Ciletuh Geopark, West Java, Indonesia. A. Kebun Jati Selagedang, B. Kebun Kelapa Tamanjaya, C. Curug Awang Atas

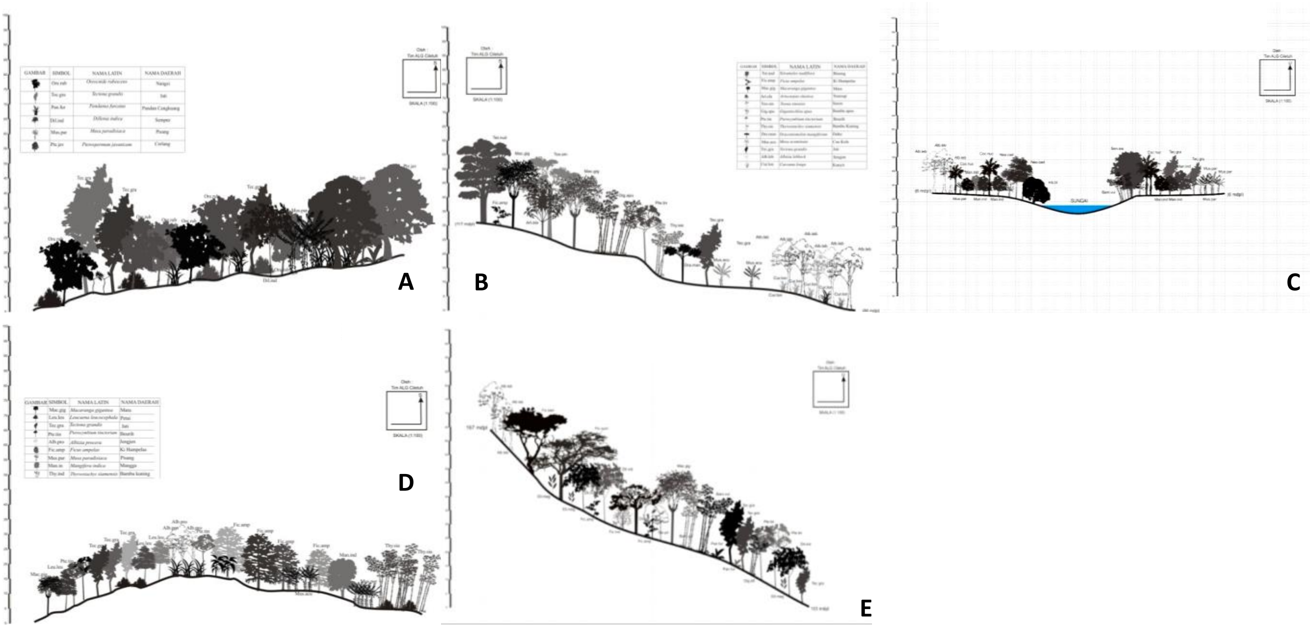

Figure 7. Vegetation profile diagram of agroforest (*talun/kebon tatangkalan*) in Ciletuh Geopark, West Java, Indonesia. A. Cigembong, B. Cipeucang Bawah, C. Mandrajaya-Ciwaru, D. Pasir Muncang, E. Gunung Masigit

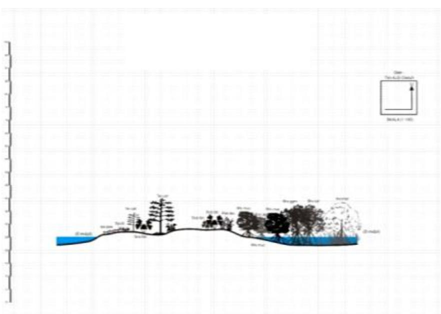

Figure 8. Vegetation profile diagram of mangrove in Ciletuh Geopark, West Java, Indonesia, i.e., Mangrove Cikadal.

Vegetation composition

Vegetation inventory recorded 179 species across all types of vegetations. Family with the highest number of species were Fabaceae and Moraceae with 13 species. The forests in Ciletuh Geopark had high species diversity compared to garden and agroforest. The most dominant species were from family Moraceae, such as *Ficus ampelas* Burm.f. and *Ficus rostrata* Thunb.

In contrast to forest and agroforest, the vegetation of garden and coastal and mangrove was different. In the garden, there were various agricultural crops species while vegetation on the beach included *Calophyllum inophyllum* and *Terminalia catappa*, whereas the dominant species were *Ipomoea pes-caprae*, and *Sesuvium portulacastrum*. Meanwhile, the dominant species of mangrove were *Avicennia marina*, *Rhizophora mucronata*, *Excoecaria agallocha*, and *Bruguiera gymnorrhiza*.

Table 1. List of species recorded in Ciletuh Geopark, West Java, Indonesia

Family	Species
Acanthaceae	*Acanthus ilicifolius* L.
Aizoaceae	*Sessuvium portulacastrum* (L.) L.
Amaryllidaceae	*Crinum asiaticum* L.
Anacardiaceae	*Anacardium occidentale* L.
Anacardiaceae	*Dracontomelon mangiferum* Blume
Anacardiaceae	*Gluta renghas* L.
Anacardiaceae	*Gluta wallichii* (Hook.f.) Ding Hou
Anacardiaceae	*Mangifera foetida* Blume
Anacardiaceae	*Mangifera indica* L.
Anacardiaceae	*Spondias dulcis* Forst.f.
Annonaceae	*Orophea hexandra* Blume
Apocynaceae	*Alstonia scholaris* (L.) R. Br.
Araceae	*Alocasia longifolia* Engl. & K. Krause
Araceae	*Colocasia esculenta* Schott
Arecaceae	*Aegle marmelos* (L.) Corrêa
Arecaceae	*Arenga obtusifolia* Mart.
Arecaceae	*Arenga pinnata* (Wurmb) Merr.
Arecaceae	*Calamus viminalis* Willd
Arecaceae	*Caryota rumphiana* Mart.
Arecaceae	*Cocos nucifera* Linn.
Arecaceae	*Daemonorops melanochaetes* Blume
Arecaceae	*Pinanga coronata* (Blume ex Martelli) Blume
Arecaceae	*Plectocomia elongata* L.
Asparagaceae	*Cordyline fruticosa* (L.) A.Chev.
Aspleniaceae	*Asplenium nidus* L.
Asteraceae	*Ageratum conyzoides* (L.) L.
Asteraceae	*Chromolaena odorata* (L.) R.M.King & H.Rob.
Asteraceae	*Crassocephalum crepidiodes* (Benth.) S. Moore
Asteraceae	*Erigeron sumatrensis* Retz.
Asteraceae	*Mickania cordata* (Burm.f.) B.L.Rob.
Asteraceae	*Sonchus arvensis* L.
Asteraceae	*Wedelia triloba* (L.) Hitchc.
Averrhoaceae	*Averrhoa bilimbi* L.
Avicenniaceae	*Avicennia marina* (Forssk.) Vierh.
Balsaminaceae	*Impatiens platypetala*
Bambusaceae	*Bambusa vulgaris* Schrad.
Bambusaceae	*Dendrocalamus asper* (Schult.) Backer
Bambusaceae	*Gigantochloa apus* (Schult.) Kurz
Bambusaceae	*Gigantochloa atroviolacea* Widjaja
Bambusaceae	*Gigantochloa atter* (Hassk.) Kurz
Bambusaceae	*Thyrsostachys siamensis* Gamble
Begoniaceae	*Begonia robusta* Blume
Bignoniaceae	*Oroxylum indicum* (L.) Kurz
Bombacaceae	*Durio zibethinus* Murr
Caricaceae	*Carica papaya* L.
Clusiaceae	*Calophyllum inophyllum* L.
Combretaceae	*Terminalia catappa* L.
Convolvulaceae	*Ipomoea pes-caprae* (L.) R. Br.
Cycadaceae	*Cycas rumphii* Miq.
Dilleniaceae	*Dillenia indica* L.
Dilleniaceae	*Tetracera scandens* (L.) Merr.
Dioscoreaceae	*Dioscorea hispida* Dennst.
Ebenaceae	*Diospyros pilosanthera* Blanco
Euphorbiaceae	*Acalypha lanceolata* Willd.
Euphorbiaceae	*Euphorbia hirta* L.
Euphorbiaceae	*Excoecaria agallocha* L.
Euphorbiaceae	*Macaranga gigantea* Rchb.f. & Zoll.) Müll.Arg.
Euphorbiaceae	*Manihot esculenta* Crantz
Fabaceae	*Acacia auriculiformis* Benth.
Fabaceae	*Acacia mangium* Willd.
Fabaceae	*Albizia lebbeck (*Osbeck). Merr.
Fabaceae	*Albizia procera* (Roxb.) Benth.
Fabaceae	*Albizzia lebbeck* L.
Fabaceae	*Archidendron pauciflorum* (Benth.) I.C.Nielsen
Fabaceae	*Calliandra calothyrsus* Meisn
Fabaceae	*Callotropis gigantea* (Hook.) G.Don
Fabaceae	*Cynometra ramiflora* L.
Fabaceae	*Erythrina variegata* L.
Fabaceae	*Gliricidia sepium* (Jacq.) Walp.
Fabaceae	*Gliricidia sepium* L.
Fabaceae	*Milletia elliptica* (Roxb.) Steud.
Fabaceae	*Mimosa pigra* L.
Fabaceae	*Mimosa pudica* L.
Fabaceae	*Parkia speciosa* Hassk.
Fabaceae	*Parkia speciosa* Hassk.
Fabaceae	*Pueraria phaseoloides* (Roxb.) Benth.
Fabaceae	*Senna siamea* (Lam.) H.S.Irwin & Barneby
Fabaceae	*Tamarindus indica* L.
Goodeniaceae	*Scaevola taccada* (Gaertn.) Roxb.
Hypoxidaceae	*Molineria capitulata* (Lour.) Herb.
Lamiaceae	*Clerodendrum laevifolium* Blume
Lamiaceae	*Tectona grandis* Linn.f.
Lythraceae	*Lagerstroemia speciosa* (L.) Pers.
Malvaceae	*Ceiba petandra* Gaertn.
Malvaceae	*Grewia laevigata* Vahl.
Malvaceae	*Heritiera* sp.
Malvaceae	*Hibiscus rosa-sinensis* L.
Malvaceae	*Hibiscus tiliaceus* L.
Malvaceae	*Melochia umbellata (*Houtt.) Stapf
Malvaceae	*Pterocymbium tinctorium* Merr.
Malvaceae	*Pterospermum javanicum* Jungh.
Malvaceae	*Thespesia populnea* (L.) Sol. ex Corrêa
Melastomataceae	*Clidemia hirta* (L.) D. Don
Melastomataceae	*Melastoma malabatrihcum* Jack.
Meliaceae	*Aglaia teysmanniana* (Miq.) Miq.
Meliaceae	*Dysoxylum alliaceum* (Blume) Blume
Meliaceae	*Dysoxylum caulostachium* (Blume) Blume
Meliaceae	*Lansium parasiticum* (Osbeck) K.C.Sahni & Bennet
Meliaceae	*Swietenia macrophylla* King
Meliaceae	*Swietenia mahagoni* (L.) Jacq.
Meliaceae	*Toona sinensis* R. Roem
Moraceae	*Artocarpus altilis* (Parkinson) Fosberg
Moraceae	*Artocarpus elastica* Roxb.
Moraceae	*Ficus ampelas* .Burm.f.
Moraceae	*Ficus ampelas* Burm.f.
Moraceae	*Ficus benjamina* L.
Moraceae	*Ficus fistulosa* Reinw. ex Blume
Moraceae	*Ficus grossularioides* Burm.f.

Moraceae	*Ficus lepicarpa* Blume
Moraceae	*Ficus rostrata* Thunb.
Moraceae	*Ficus septica* Burm.f.
Moraceae	*Ficus sumatrana* Miq.
Moraceae	*Ficus variegata Blume*
Moraceae	*Parartocarpus venenosa* Becc.
Moraceae	*Stelechocarpus burahol* (Blume) Hook.f. & Thomson
Muntingiaceae	*Muntingia calabura* L.
Musaceae	*Musa acuminata* Colla.
Musaceae	*Musa x paradisiaca* L.
Myrtaceae	*Psidium guajava* L.
Myrtaceae	*Syzygium aromaticum* (L.) Merr. & L.M.Perry
Myrtaceae	*Syzygium densiflorum* Wall. ex Wight & Arn.
Myrtaceae	*Syzygium jambos* (L.) Alston
Myrtaceae	*Syzygium lineatum* (DC.) Merr. & L.M.Perry
Pandanaceae	*Galearia filiformis* Boerl.
Pandanaceae	*Pandanus furcatus* Roxb.
Pandanaceae	*Pandanus tectorius* Parkinson ex Du Roi
Passifloraceae	*Passiflora foetida* L.
Phyllanthaceae	*Breynia racemosa* (Blume) Müll.Arg.
Phyllanthaceae	*Cleistanthus monoicus* (Lour.) Müll.Arg.
Phyllanthaceae	*Phyllanthus emblica* L.
Piperaceae	*Piper aduncum* L.
Piperaceae	*Piper betle* L.
Poaceae	*Spinifex littoreus* (Burm. f.) Merr.
Poaceae	*Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb.
Poaceae	*Cymbopogon citratus* (DC.) Stapf
Poaceae	*Imperata cylindrica* (L.) Raeusch.
Poaceae	*Oryza sativa* L.
Poaceae	*Oryza sativa* var. x
Poaceae	*Panicum maximum* Jacq.
Poaceae	*Pennisetum polystachion* (L.) Schult.
Poaceae	*Saccharum spontaneum* L.
Poaceae	*Zea mays* L.
Polygalaceae	*Xanthophyllum excelsum* (Blume) Miq.
Rafflesiaceae	*Rafflesia patma* Blume.
Rhizophoraceae	*Bruguiera cylindrica* (L.) Blume
Rhizophoraceae	*Bruguiera gymnorrhiza* (L.) Lamk
Rhizophoraceae	*Carallia brachiata* (Lour.) Merr.
Rhizophoraceae	*Rhizophora mucronata* Lam.
Rubiaceae	*Guettarda speciosa* L.
Rubiaceae	*Morinda citrifolia* L.
Rubiaceae	*Neolamarckia cadamba* (Roxb.) Bosser
Rubiaceae	*Neonauclea lanceolata* (Blume) Merr.
Rutaceae	*Acronychia pedunculata* (L.) Miq.
Rutaceae	*Citrus aurantiifolia* (Christm.) Swingle
Rutaceae	*Citrus hystrix* DC.
Rutaceae	*Melicope latifolia* (DC.) T.G. Hartley
Sapindaceae	*Filicium decipiens* (Wight & Arn.) Thwaites
Sapotaceae	*Chrysophyllum cainito* L.
Sapotaceae	*Manilkara zapota* (L.) P.Royen
Sapotaceae	*Palaquium rostratum* (Miq.) Burck
Sterculiaceae	*Sterculia foetida* L.
Tetramelaceae	*Tetrameles nudiflora* R.Br.
Urticaceae	*Oreocnide rubescens* Blume
Verbenaceae	*Lantana camara* L.
Verbenaceae	*Stachytarpheta jamaicensis* Gardn
Zingiberaceae	*Alpinia galanga* (L.) Willd.
Zingiberaceae	*Costus speciosus* (J.Konig) Sm.
Zingiberaceae	*Curcuma longa* L.
Zingiberaceae	*Etlingera megalocheilos* (Griff.) A.D.Poulsen
Zingiberaceae	*Hedychium roxburghii* Blume
Zingiberaceae	*Zingiber officinale* Roscoe

Note: 7 samples have not been identified yet

Discussion

In Ciletuh Geopark there has been changes in land use in which agricultural land was more dominant than forest. The existing forest vegetation was only remained in few areas, meaning that previously Ciletuh Geopark had experienced deforestation. Deforestation is the conversion of forest areas to non-forest land use (Gervet, 2007). Agriculture is one of the most significant causes of deforestation (Bennett, 2017). Effect deforestation includes the reduction or even loss of native species replaced by cultivated species. Deforestation can be caused by several factors including population pressures, commercial activities, and social and political conditions.

Currently, Ciletuh Geopark is dominated by *talun/ kebon tatangkalan*. *Talun* is an agroforestry system formed to increase overall productivity and serve various functions by combining agricultural crops with tree vegetation (Berkes 2012; Parikesit et al. 2005). *Kebon tatangkalan* has distinctive features and has developed under the influence of various biophysical and socio-economic factors (Parikesit et al. 2005).

Soerianegara and Indrawan (2005) state natural plant communities in tropical forests have at least three strata. Although the Ciletuh Geopark was dominated by trees from strata B and C, but the forest in the Puncak Manik area was still dominated by trees from strata A. Vegetation in Ciletuh Geopark were arranged of trees from strata A, B, and C, indicating the existence of old plants (Suci et al. 2017). The findings of this study highlight the importance of conserving the remaining natural vegetation in Ciletuh Geopark to enhance the biological values of the geopark.

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REFERENCES

Bennett L. 2017. Deforestation and Climate Change. Climate Institute. Washington DC.

Berkes F. 2012. Sacred Ecology. Routledge. New York.

Gem C. 1996. Kamus Saku Biologi. Erlangga. Jakarta.

Gervet B. 2007. Deforestation Contributes to Global Warming. Luleå University of Technology. Luleå, Sweden.

Megantara EN. 2016. Biodiversity Ciletuh: Sekilas Diversitas Hayati & Sebaran REEPS (Rare, Endangered, Endemic, & Protected Species). Unpad Press. Bandung. [Indonesian]

Misra R. 1973. Ecology Work Book. Oxford and IBH Publishing Co. New Delhi.

Mueller-Dombois D, Ellenberg H. 1974. Aims and Methods of Vegetation Ecology. John Wiley & Sons, New York.

Parikesit, Takeuchi K, Tsunekawa A, Abdoellah OS. 2004. Kebon Tatangkalan: A disappearing agroforest in the Upper Citarum Watershed, West Java, Indonesia. Agrofor Syst 63 (2): 171-182. DOI: 10.1007/s10457-004-1182-x

Richard PW. 1966. The Tropical Rain Forest an Ecological Study. Cambridge University Press, London.

Soerianegara I, Indrawan A. 2005. Ekologi Hutan Tropika. Bogor : Laboratorium Ekologi Hutan Fakultas Kehutanan Institut Pertanian Bogor. Bogor.

Suci, Dahlan Z, Yustian I2017. Propil vegetasi di kawasan hutan konservasi Suaka Margasatwa Gunung Raya Kecamatan Warkuk. Jurnal Penelitian Sains 19 (1): 47-53. [Indonesian]

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Analysis of climate change knowledge and its implications on livelihood options around Naituyupaki Location, Maasai Mau Forest, Narok County, Kenya

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Abstract. *Kong'ani LNS, Mutune JM, Thenya T. 2018. Analysis of climate change knowledge and its implications on livelihood options around Naituyupaki Location, Maasai Mau Forest, Narok County, Kenya. Asian J For 2: 62-66.* Climate change knowledge among rural communities adjacent to forests influences their response in terms of mitigations and adaptations of their livelihoods. Rural households are highly dependent on natural resources, whose base is highly indisputably threatened by the changing climate. The aim of this study was to reveal the knowledge on climate change perceived by forest-based communities around Maasai Mau Forest, Narok County, Kenya. Results indicate that 93% of the respondents were aware of climate change through life experiences. The cited primary causes of climate change by 72% of the respondents included natural causes (26%), human activities (2%) and punishment from Gods respectively. The rural communities also perceived that their main livelihood activities (i.e., agricultural production and forest products collection) were to a large extent affected by climate change. There is a need to continuously expose local community to emerging knowledge on the impact of climate change for improved mitigation and adaptations.

Keywords: Climate change, implications, knowledge, livelihoods options, mitigation

INTRODUCTION

Most rural households rely on a diverse portfolio of activities and income sources in which crop and livestock production play important contributions to household well-being. These livelihoods support systems are, however, threatened by the changing climate that is characterized by more frequent floods, prolonged drought, erratic rainfall patterns, emerging disease, and pest incidences. This scenario particularly affects communities that live adjacent to forests. Altogether with climate change, these forests have already been pressured by increased demand for new agricultural lands, dependence on forest grazing, incidences of charcoal burning, illegal logging, and demand for non-timber forest products to supplement livelihoods support gaps (GoK 2012).

While potentially adversely affecting rural communities, knowledge of climate change among such communities remains low in spite of highly publicized debate. For example, research by Dube and Phiri (2013) indicated low awareness in Zimbabwe. Similar observations were made by Onyekuru et al. (2014) on rural livelihoods in Nigeria. While studies by Boon and Ahenkan (2012) in Ghana Sui Forest Reserve, and Tambo and Abdoulaye (2013) in Nigeria savanna noted that forest provides vital resources to enable rural communities to cope with climate change.

Kenya currently has forest cover of only 6%, much lower than the Kenyan constitutional requirement of 10% (FAO 2010; GoK 2010; Kimutai and Watanabe 2016). In Kenya, there is limited documentation on the knowledge of climate change among communities that live adjacent to forest ecosystems. It is notable that most of the studies on climate change impact on livelihood particularly in Kenya are biased towards arid and semi-arid lands (ASALs) (Macharia et al. 2012; Ogalleh et al. 2012).

The aim of this research was to document and provide empirical evidence on the knowledge on climate change among the forest adjacent communities in Maasai Mau ecosystem, Kenya and how this affects their livelihood dynamics.

MATERIALS AND METHODS

Study area

This study was carried out in a rural community around Maasai Mau Forest, Naituyupaki, Narok County, Kenya (Figure 1). The community-managed natural forest with an extent of 46,278 hectares. The forest comprises large stands of cedar (*Juniperus procera)* and Podo (*Podocarpus gracillor*) with scattered natural glades (Thenya and Kiama 2008). It has a high diversity of fauna including the rare mountain Bongo (*Tragelaphus eurycerus*). This forest forms the southern part of the Mau forest complex, Kenya's largest closed-canopy forest area. Maasai Mau forms the catchment of Ewaso Ngiro and Mara rivers that supply important water to the region like Lake Natron and world-famous Maasai Mara ecosystem.

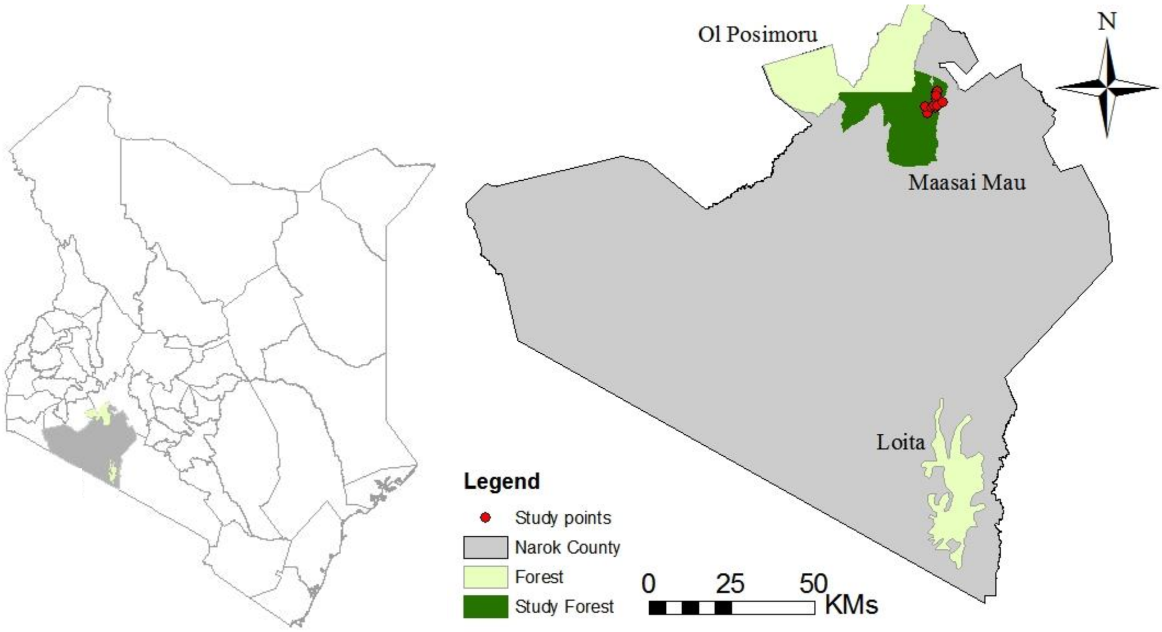

Figure 1. Map of the study location in the Maasai Mau forest, Naituyupaki-Olokurto, Kenya

The area around Maasai Mau forest is characterized by bimodal rainfall pattern that ranges between 1,000 to 2,000mm per annum. The temperatures range from 16°C to 22°C with July being the coldest month. The soils are deep, well-drained, fine-textured and of high agricultural potential (Kinyanjui 2009).

The study area Naituyupaki-Olokurto covered an area of about 527.60 km^2 with a population of approximately 21,045 and an estimated 3,811 households (Kenya National Bureau of Statistics 2010). The forest supports the local communities in terms of building materials, wood fuel, charcoal, herbs, pasture, fruits, honey, water and also provides an important site for spiritual and cultural purposes (Thenya and Kiama 2008; GoK 2012).

Study design, sampling and data collection

The study was conducted in the month of June 2016 in Naituyupaki, Narok County with a focus on forest adjacent communities. Naituyupaki had a total of seven villages including Naituyupaki, Sauli, Ndete, Legen/Sasimueni, Nalengoi, Esoitit, and Sagatia. Data was, however, collected in Legen/Sasimueni, Nalengoi, Esoitit and Sagatia villages, which were easily accessible. The four villages were largely dominated by the Ogieks, Maasai's and Kikuyus ethnic groups. Factors that restricted access to Naituyupaki, Sauli and Ndete villages' access included long-distance, difficult terrain, and uncooperative communities' as learned during the reconnaissance study.

The study applied blended methodologies to collect quantitative and qualitative data. From a total of 183 households in the seven villages, a sample size of 53 households was calculated at 95% confidence level as follows (N=183).

$n = (z^2 \times p \times q \times N)$

$e^2 (N - 1) + (z^2 \times p \times q);$

where: n = Sample size (being determined), N = Population size (which is known), p = Sample proportion (assumed to be 0.05, if not given), q = 1 - p, e = 0.05 (since the acceptable error (level of significance) should be 5%) and z = Standard deviation at a given CI (z = 1.96 at 95% CI). Based on the available household data, the 53 households were proportionately distributed as follows (Table 1).

The household survey was conducted using structured questionnaire that was administered to the 53 household heads with the help of four local research assistants among whom one was a village elder. The respondents were systematically selected from a list of households picking every 4th household in each of the respective villages to ensure equitable representation. To ensure equal representation of both male and female, a list was drawn indicating male and female-headed households and used for picking respondents systematically according to the proportionate villages' sample.

Table 1. Sample size distribution across four villages

Village	Total no. of households	Calculated sample size
Legen/Sasimueni	38	11
Nalengoi	55	16
Esoitit	31	9
Sagatia	59	17
Total	183	53

The household survey information was triangulated with participatory assessments and field observations. Participatory assessments included two Focus Group Discussions (FGDs) conducted at each village, each comprised seven participants. The participants were randomly selected among the households. The checklist used to guide the FGD was prepared using information generated from the questionnaire survey. Key informant interviews were also conducted with the Ministry of Environment, Water, Energy and Naturals resources (MEWENR), Kenya Forest Service (KFS) - Ecosystem Conservancy, village elder and Community Forest Association (CFA) officials. The key informants were purposely selected for their ability to inform study objectives. A checklist was used for the key informant interviews. In all the interviews, no consent was given to voice record the interviews and therefore meticulous notes were taken. Field transect walk, observation, informal talks with research assistants and field contact person were also executed as an additional method to triangulate information gathered. The qualitative data were analyzed using thematic approach while quantitative data was analyzed using descriptive statistics.

RESULTS AND DISCUSSIONS

Livelihood activities

The respondents in the study area were found to depend on a variety of livelihood activities with the most important being crop production, livestock production and extraction of forests products. Other livelihood activities included petty trading and casual laborers. The household survey results indicated that 96% of the respondents mainly grew maize besides beans, peas, carrots, potatoes, cabbages and tree tomatoes. More than half of the households grew at least two types of these crops. About 14% of the respondents raised livestock either as their main livelihood activity, which included cattle, sheep, goats, donkeys, and chicken. All the respondents were found to rely heavily on forests for firewood, charcoal, building materials, honey, and fruits. Agricultural production in the area was purely rain-fed, which rendered the communities more vulnerable to changes in climate.

Knowledge of climate change

Results from the household survey indicated that 93% of the respondents had heard or knew about climate change, which was confirmed in the FGDs where most of the participants held the same opinion. About 43% of the respondents had experienced climate change effect on livelihood disruption. Some of the sources of information on climate change according to about 23% of the respondents were via radio, public meetings, and hear-say from other farmers, agricultural extension agents and seminar.

A number of observed evidence and understanding of climate change in the area was noted as freezing morning hours, prolonged hot/dry period, and reduced rainfall than earlier years. Others were increased worry and observation of crop failure each season due to unpredictable rainfall patterns, and increased diseases and pest attacks on crops leading to food insecurity. Flash floods and prolonged drought were reported to have become more severe. The farming seasons had changed from what the communities knew with increased flash floods, prolonged and frequent droughts leading to livestock mortality and crop losses.

Similar findings were documented by Egbe et al. (2014) in Nigeria where local community had made similar observations. In Kenya, a study by Kuria (2009)'s in Kereita forest recorded that local community knowledge and perception of climate change was high at 87%. Dube and Phiri (2013) study in Matobo and in other parts of Zimbabwe also found that about 51% of their respondents had never heard about climate change, in contrast to the findings in the Maasai Mau forest where 93% had heard of climate change. This demonstrates the probability of inadequate information and knowledge among some communities although they were aware of the significant changes taking place in their environment. Like in Zimbabwe, report by Muhumuza et al. (2011) in Rwenzori region suggested that climate change subject was not well known to most of the respondents and particularly to those with less exposure to external knowledge.

Causes of climate change

About 72% of the respondents cited natural causes as the primary cause of climate change, while 26% and 2% cited human activities and punishment from Gods respectively. This result agrees with Debela et al. (2015), which found that 78% of the respondents in South Ethiopia mentioned natural forces as the primary cause of climate change and 16% cited human activities. The results also corroborate the findings of Caribbean Institute of Media and Communication (2012) report on climate change knowledge, attitude and behavioral practice survey, which observed that 43% of the respondents cited natural causes. The respondents' perception of natural causes as the primary cause of climate change may have been contributed by the communities' lack of adequate information and knowledge about the scientific understanding of what natural causes are. According to Nwankwoala (2015), the natural causes of climate change included volcanic eruptions, solar radiations, biotic processes and even ocean currents, which is contrary to the likely understanding of forest adjacent communities in Maasai Mau ecosystem.

Participant observation during transect walks recorded anthropogenic activities, including charcoal burning and clearance of forest to pave way for crop production in Maasai Mau, that could have contributed to prolonged drought spells, floods, erratic rainfall patterns, increased pests, and diseases incidences. Some of the respondents either lived in the forest land or were connected to people living in the forest. Thus, the respondents might have concealed information as regards their activities that could be attributed to decreased forest ecosystem changes for fear of eviction. Scientific findings by the IPCC, 5AR (2014), demonstrated that human activities are the primary cause of climate change as observed in the study area. The communities depend largely on their own observations and experiences as sources of information as noted by 93% who had observed changes taking place in the environment, which is inadequate. The respondents' knowledge of the primary cause of climate change is still limited as access to such information is limited. This finding strengthens the GoZ-UNDP/GEF (2010)'s report, which suggested that most of the people in the less developed countries are not adequately informed of climate change. Climate change information is largely limited to research institutions in these countries coupled with little dissemination. In Maasai Mau forest, only 2% of the respondents were reported to have received training related to climate change from the Government's extension officers thus demonstrating limited efforts in dissemination of climate change information among the FACs. The communities' strategies in dealing with any climate-induced risks and opportunities are undeniably influenced by their knowledge on the same as also observed by Bryan et al. (2009) and Komba and Muchapondwa (2012) in Ethiopia and Tanzania, respectively. The acquired knowledge contributed largely in determining what livelihood practices communities adapt to climate change as also observed by Nzeadibe et al. (2011) findings in the Niger Delta region of Nigeria. It is likely that the communities' knowledge of climate change as established will stagnate and or even be lost if it is not aligned with scientific knowledge thus increasing communities' vulnerability to climate change impacts.

According to Ozor et al. (2015) observation regarding perceived punishment from Gods as a cause of climate change is common in most rural areas. Some communities across the globe believe that some evil deeds trigger Gods' wrath that can alter the normal climatic patterns thus affecting livelihoods activities as also observed by 2% of the respondents in the study area, thus suggesting low links between climate change causes to perceived Gods punishment among communities.

Climate change implications on livelihood activities

All the respondents indicated that they had experienced climatic variations in the past ten years, which had consequently affected their livelihoods. The communities attributed the changing climate to increased drought incidences (15%), drying up of streams (8%), irregular rainfall patterns (6%) and increased pests and diseases occurrences (37%) that all resulted in poor crop yields (34%). Notably, pests and disease incidences caused crop failure rendering the communities more vulnerable to food insecurity in the study area. According to Hartter et al. (2012), for the local communities in most of the tropics, precipitation bears much significance regarding impacts as compared to temperature changes. The change in rainfall quantity and distribution pattern affects agricultural production and forest conditions as also observed in the study area, which is also likely to continue while impinging on communities livelihoods.

The findings by Onyekuru et al. (2014) in Nigeria observed that diseases incidences are linked to declining agricultural crop production. Similarly, Agrawala et al. (2003) study in Tanzania found that the growing of crops such as maize, finger millets, beans, cowpeas, and groundnuts was reported to have declined following the effects of increased incidences of pests, diseases, and vermin. According to the IPCC's Fifth Assessment Report, global agricultural production has already declined by 1-5% per decade as result of climate change effects (IPCC 2014). Dinesh et al. (2015) noted that crop pests, exacerbated by the rise in temperatures, are already a major factor influencing farm productivity in the continent, and about one-sixth of the field production is lost to pests. This confirms the fears of the communities in the study area as they cited increased incidences of diseases and pests as a sign of the changing climate and food crop losses that are likely to increase food insecurity among the households.

In conclusion, the Maasai Mau Forest Community had some knowledge on climate change that they believed was primarily caused by their own activities including charcoal burning, logging, and clearing of forest for agricultural production. At the same time, it is noted that rural communities have limited access to formal means of gaining climate change information. As the effects of climate change are likely to increase over time, there are high chances that communities around Maasai Mau forest are likely to experience more vulnerability to their livelihood. Therefore, government and civil society organizations should increase access to information on climate change and customization of scientific data on climate change that could help the local communities participate in mitigation and adaptation of climate change accordingly. Such action could be facilitated through agricultural extension systems linking livelihood and climate change information that is currently missing.

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REFERENCES

Agrawala S, Moehder A, Hemp A, Van Aalst M, Hitz S, Smith J, Meena H, Mwakifwamba S, Hyera T, Mwaipopo O. 2003. Development and Climate Change in Tanzania: Focus on Mount Kilimanjaro. OECD, Paris.

Boon E, Ahenkan A. 2012. Assessing climate change impacts on ecosystem services and livelihoods in Ghana: case study of communities around Sui Forest Reserve. J Ecosyst Ecogr S3:001. DOI:10.4172/2157-7625.S3-001.

Bryan E, Deressa TT, Gbetibouo GA, Ringler C. 2009. Adaption to climate change in Ethiopia and South Africa: options and constraints. Environ Sci Pol 12 (4): 413-426. DOI: 10.1016/j.envsci.2008.11.002

Caribbean Institute of Media and Communication. 2012. Report on Climate Change Knowledge, Attitude and Behavioral Practice Survey. University of the West Indies at Mona, Jamaica.

Debela N, Mohammed C, Bridle K, Corkry R, McNeil D. 2015. Perception of climate change and its impact by smallholders in pastoral/agropastoral systems of Borana, South Ethiopia. SpringerPlus 4: 236. DOI: 10.1186/s40064-015-1012-9.

Dinesh D, Randall-Boone B, Delia G, Kinyangi J, Lindahl J, Vishnumurthy C.M, Ramirez-Villegas J, Robinson T, Rosenstock T, Smith J, Thornton P. 2015. Impact of climate change on African agriculture: focus on pests and diseases. CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS), Copenhagen, Denmark.

Dube T, Phiri K. 2013. Rural livelihoods under stress: The impact of climate change on livelihoods in South Western Zimbabwe. Amer Intl J Contemp Res 3 (5): 11-25.

Egbe A, Yaro MA, Okon AE, Bisong FE. 2014. Rural peoples' perception of climate variability/change in Cross River State-Nigeria Cyprian. J Sustain Dev 7 (2): 25-36. DOI:10.5539/jsd.v7n2p25

FAO. 2010. Global Forest Assessment. Food and Agricultural Organization of the United Nation, Rome, Italy.

GoK. 2010. Government of Kenya, Constitution of Kenya, National Council for Law Reporting.

GoK. 2012. Naituyupaki Zonal Management Plan. Maasai Mau Ecosystem, Naituyupaki-Olokurto, Kenya.

GoZ-UNDP/GEF. 2010. Coping with drought and climate change project. Capacity needs assessment and strategy for enhanced use of seasonal climate forecasts for smallholder farmers in Chiredzi District. Final Report. GoZ-UNDP/GEF, New York.

Hartter J, Stampone M.D, Ryan S.J, Kriner K, Chapman C.A, Goldman A. 2012. Patterns and perceptions of climate change in a biodiversity conservation hotspot. PLoS ONE 7(2): e32408. DOI: 10.1371/journal.pone.0032408

Kenya National Bureau of Statistics. 2010. 2009 Kenya Population and Housing Census, Volume I.A. Population Distribution by Administrative Units. Ministry of Planning and National Development, Nairobi, Kenya.

Kimutai KD, Watanabe T. 2016. Forest-Cover Change and Participatory Forest Management of the Lembus Forest, Kenya. Environments 3(3): 20. DOI:/10.3390/environments3030020

Kinyanjui JM. 2009. Case study on changes in NDVI values in the period 1998 - 2009: the case of Maasai Mau, South-western Mau and Eastern Mau. DRSRS, Nairobi, Kenya

Komba C, Muchapondwa E. 2012. Adaptation to Climate Change by Smallholder Farmers in Tanzania. Economic Research Southern Africa (ERSA), Cape Town.

Kuria D. 2009. Coping with climate change: Understanding local communities' knowledge and their coping strategies to climate change. Birdlife International. Nairobi, Kenya.

Macharia PN, Thuranira EG, Ng'ang'a LW, Lugadiru J, Wakori S. 2012. Perceptions and adaptation to climate change and variability by immigrant farmers in semi-arid regions of Kenya. African Crop Sci J 20 (Supplement s2): 287-296.

Muhumuza M, Muzinduki P, Hyeroba G. 2011. Small Holder Farmers Knowledge and Adaptation to Climate Change in the Rwenzori Region. Rwenzori J 1:111-133.

Nwankwoala HNL. 2015. Causes of climate and environmental changes: the need for environmental-friendly education policy in Nigeria. J Educat Pract 6 (30): 224-234.

Nzeadibe TC, Egbule CL, Chukwuone NA, Agu VC. 2011. Farmers' Perception of Climate Change Governance and Adaptation Constraints in Niger Delta Region of Nigeria. African Technology Policy Studies Network Nairobi, Kenya.

Ogalleh SA, Vogl CR, Eitzinger J, Hauser M. 2012. Local Perceptions and Responses to Climate Change and Variability: The Case of Laikipia District, Kenya. Sustainability 4: 3302-3325. DOI: 10.3390/su4123302

Onyekuru AN, Marchant R. 2014. Climate change impact and adaptation pathways for forest-dependent livelihood systems in Nigeria. African J Agric Res 9 (24): 1819-1832.

Ozor N, Umunake PC, Ani AO, Nnadi FN. 2015. Perceived impacts of climate change among rural farmers in Imo State, Nigeria. African J Agric Res 10 (14): 1756-1764.

Tambo JA, Abdoulaye T. 2013. Smallholder farmers' perceptions of and adaptations to climate change in the Nigerian savanna. Reg Env Chang 13 (2):375-388. DOI: 10.1007/s10113-012-0351-0

Thenya T, Kiama S. 2008. Socio-economic survey and livelihood analysis of the Maasai Mau Ecosystems. Technical series, COMIFORM Project Kenya Forest Working Group, Kenya.

Thenya T. 2014. Forest Based Income Generating Potential (IGP) high community expectations amidst low community transformation; an analysis of PFM implementation between 2005 and 2013. Proceedings of the Second National PFM Conference: Enhancing participatory forest management under the devolved governance structure. Kenya Forestry Research Institute, Muguga, Kenya.

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Ice Nucleation Active bacteria in Mount Lawu forest, Indonesia: 3. Isolation and estimation of bacterial populations on bryophyte

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Abstract. *Latifah NH, Susilowati A, Suratman. 2018. Ice Nucleation Active bacteria in Mount Lawu forest, Indonesia: 3. Isolation and estimation of bacterial populations on bryophyte. Asian J For 2: 67-75.* This study aimed to isolate and estimate the Ice Nucleation Active (INA) bacteria population on bryophyte from the trekking route of Cemoro Sewu, Mount Lawu, Indonesia. Bryophyte samples were taken at three different altitudes, i.e., 2,000, 2,300, and 2,500 m above sea level (asl). Isolation of INA bacteria was carried out by the method of spread plate on King's B and nutrient agar plus 2.5% glycerol (NAG) medium. Bryophyte was identified by referring to the literature of Sutama (1995), Hasan and Ariyanti (2004), Ignatova and Samkova (2006), and Damayanti (2006). Ice nucleation activity was determined by the multiple-tube test method. The bacterial suspension tube was put into the circulating alcohol bath at a temperature of -10°C for 5 minutes. The number of INA bacteria on the bryophyte was estimated with the nucleation multiple tube method. The number (cell/g) of INA bacteria from the fresh weight of bryophyte was estimated based on the Most Probable Number (MPN) tables according to the formula of Thomas's 333 series. INA bacteria were identified through morphological and biochemical characters. Independent Sample T-Test analyzed the population of INA bacteria on terrestrial and epiphytes bryophytes with a significance of 5%. The results showed that 7 INA bacteria were isolated from the bryophyte *Campylopus umbellatus* (Arn.) Paris (Leucobryaceae, Musci). The population of INA bacteria in terrestrial bryophytes at each altitude were greater with 346 cell/g, 86 cell/g, and 396 cell/g, respectively, than that in epiphytes bryophyte with 50 cell/g, 50 cell/g, 176 cell/g, respectively.

Keywords: Bryophyte, Ice Nucleation Active, INA, isolation, Lawu, population

INTRODUCTION

The number of bacteria in nature is very large and diverse in terms of morphology, physiology, and genetics. Even in different habitats, the diversity of growing bacteria is also high. The bacteria that inhabit the leaf surface vary according to the plant species because each plant produces a certain exudate suitable for certain bacteria. The height of a place also affects the species of bacteria that grow on the leaves. Altitude affects the low air temperature, so only certain bacteria can adapt to this extreme environment (Morris et al. 2004). One bacterial species that grow on the leaf surface of upland plants with low air temperature is the Ice Nucleation Active (INA) bacteria (Lindow et al. 1978). INA bacteria that have been found are *Pseudomonas syringae*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Erwinia herbicola*, and *Xanthomonas campestris* pathovar *translucens* (Edwards et al. 1994). These five bacterial species can catalyze ice formation at temperatures of -1.5°C to -10°C, even at temperatures above -5°C. These species could cause frost injury on the leaf surface. It was caused by changes in the water between and inside the leaf cells into ice at a temperature of -5°C (Gurian-Sherman and Lindow 1993).

INA bacteria are bacteria capable of catalyzing ice formation at temperatures above -10°C. INA bacteria can express Ice nucleation proteins on the cell surface, lowering the water temperature and freezing it. If there is no ice nucleation, pure cold water (H_2O) can only be supercooled and will not spontaneously freeze until the temperature reaches -40°C. INA bacteria function to accelerate the ice freezing process (Stephani and Waturangi 2011).

Ice cores in INA bacteria, active at relatively warm temperatures (≥-5°C), can play an important role in climatology by helping the process of condensation and forming ice nuclei in clouds. Ice core formation in troposphere clouds is required for snow formation and most precipitation (Christner et al. 2008a,b). Ice nucleating bacteria participate in the bioprecipitation cycle, by which the bacteria move to the cloud from the surface of plant leaves carried by the wind cycle and stimulate rain. On the other hand, rain provides favorable conditions for the growth of these ice-nucleating bacteria on plant leaf surfaces (Stephanie and Waturangi 2011). Moss plants are a group of plants that have a high enough water requirement. The greatest need for water depends on rainfall and humidity in the surrounding air (Sutama 1995). Since their high water requirement, it is estimated that INA bacteria can also be found in mosses. Besides having an important role in bioprecipitation, INA bacteria can also be used to make artificial rain and snow by seeding clouds with INA bacteria as a substitute for salt sowing, which is now widely used to make artificial rain (Wahyudi 1995).

Lindow et al. (1982) research showed that INA bacteria live in low-temperature areas with an optimum temperature of around 18-24°C. Mount Lawu, Indonesia, which has an altitude of 3,265 m above sea level (asl), is a tropical mountain that is rich in moss plants, namely, those that grow terrestrial such as *Marchantia polymorpha*, *Polytrichum* sp., *Riccia* sp., or those that grow epiphytes such as *Cyathophorum bulbosum*, *Dicranoloma robustum*, and *Leptostomum inclinans*, and those that can grow terrestrial and epiphytes such as *Thuidium furfurosum* and *Dicranoloma dicarpium* (Setyawan and Sugiyarto 2001). In addition to having a variety of moss plants, Mount Lawu also has a relatively low air temperature, thus allowing a great opportunity to find ice crystal-forming phyllosphere bacteria.

Given the important role of INA bacteria in bioprecipitation and there is still little information about INA bacteria in the tropics, it is necessary to research the isolation and population number of INA bacteria from mosses in Mount Lawu. This research data can be used to understand the role of INA bacteria in bioprecipitation, which can affect cloud and rain formation.

The aims of this study were: (i) isolating INA bacteria from mosses on the trekking route of Cemoro Sewu, Mount Lawu. (ii) determining the number of INA bacteria in mosses on the trekking route of Cemoro Sewu, Mount Lawu.

MATERIALS AND METHODS

Materials

The materials to obtain INA bacteria were moss plants from the trekking route of Cemoro Sewu, Mount Lawu, East Java, Indonesia. Bacterial isolation and mosses were identified at the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia.

Procedure

Moss plant sampling

The moss plant sample consisted of one epiphytic species and one terrestrial species taken from the trekking route of Cemoro Sewu, Mount Lawu. The sampling technique used in this research is the purposive sampling method by prioritizing places that are relatively overgrown with mosses and paying attention to abiotic factors (humid environmental conditions) with no plots (Windadri 2007). The survey resulted in three sampling points with different altitudes: 2,000 m asl, 2,300 m asl, and 2,500 m asl.

The plant material was 5-10 g of moss leaves. They were put in a paper bag and labeled with information on altitude, air, soil humidity, soil pH, light intensity, and air temperature as environmental factors affecting the number of INA bacteria populations on moss plant samples. In addition, the height of the place was measured by GPS, soil pH and soil moisture were measured by a pH meter plugged into the soil where the sample was taken, a hygrometer measured humidity and air temperature, and light intensity was measured by a lux meter. During the trip to the laboratory, samples of moss plants were put in an ice box. Immediately, they were brought to the laboratory and stored in a refrigerator at a temperature of 5°C.

Moss plant identification

The identification of mosses is performed by comparing and matching the characters of mosses with the literature/references from Sutama (1995), Hasan and Ariyanti (2004), Ignatova and Samkova (2006), and Damayanti (2006).

Sterilization of tools and materials

Petri dishes, test tubes, Erlenmeyer flask, NA agar media, and other tools are sterilized first. Then, the sterilization process was carried out by wet sterilization using an autoclave with a pressure of 1 atm and a temperature of 121°C for 20 minutes.

Preparation of NA + 2.5% Glycerol (NAG), King's B, and Slant Agar Media

NA medium was prepared by mixing 6 g of NA powder with 5 mL of glycerol in a 200 mL Erlenmeyer flask and added with distilled water to a limit of 200 mL. The composition for King's B media is 20 g Proteose peptone, 10 mL glycerol, 1.5 g K_2HPO_4, 1.5 g $MgSO_4.7H_2O$ and 15 g agar put into an Erlenmeyer flask, then added with 1 L aquades (Kartika 2009). Erlenmeyer flask was placed on a hot plate and let to boil until the solution was clear. The hole in the Erlenmeyer flask was covered with cotton and aluminum foil and sterilized using an autoclave at 121°C for 20 minutes. Each NA and King's B media was poured into a petri dish and allowed to dry at room temperature (28°C).

To make the slant agar, after the solution boiled and turned clear, the NA medium was immediately poured into 4 mL test tubes and autoclaved at 121°C for 20 minutes. After sterilization, the tube was slanted at 45° until it hardened.

Isolation of INA bacteria

Each 5 g of moss leaf sample was cut into small pieces and put in a 500 mL Erlenmeyer flask containing 200 mL of 0.1 M phosphate buffer with pH 7.0 and 0.1% peptone (Difco) (Waturangi and Amelia 2009). Phosphate buffer pH 7.0 was made with 0.6 g Monosodium Phosphate (NaH_2 $PO_4.2H_2O$) and 1.6 g Disodium Phosphate Hepta Hydrate ($Na_2HPO_4.7H_2O$) for 1 L of distilled water. The Erlenmeyer flask was shaken on a rotary shaker for 2 hours at 150 rpm. After shaking, serial dilutions were carried out to reach 10^{-3} with 9 mL of sterile distilled water. A 0.1 mL of each series of dilutions was taken for spread-plating on NA + 2.5% glycerol media and King's B media and then incubated at room temperature for 24 hours (King et al. 1954).

Pure culture collection

Each colony of different bacteria that grew on NA + 2.5% glycerol media and King's B media was taken with ose and strained on NA + 2.5% glycerol media and King's B media. Then, it was incubated at room temperature for 24 hours. Next, each separate bacterial colony was streaked onto the slant agar. They were grown for 4-6 days at room temperature and then stored in the freezer at 4-5°C.

Ice nucleation activity test

Each pure culture was tested for its nucleation activity. A bacterial suspension was prepared by taking 1 ose of pure culture and diluting it in 400 µL phosphate buffer. Ice nucleation activity was determined by the multiple-tube test method by inserting a microtube containing the bacterial suspension into a circulating alcohol bath at a temperature of -10°C for 5 minutes. Frozen microtubes were colonies containing ice cores (Lindow et al. 1978).

Population number estimation of INA bacteria

Estimation of the number of INA bacteria in moss leaves was carried out by the multiple tube nucleation method. The test tube containing 9 ml of sterile phosphate buffer was cooled in an alcohol bath at -10°C for 30 minutes. The tubes were shaken, and all the frozen tubes were separated. The unfrozen tube is warmed at 5°C. Leaf moss samples in phosphate buffer solution with a ratio of 10:100, which have been shaken, are diluted 10^{-1} to 10^{-3} in series 3.3.3 tubes. After the tube was dripped, each bacterial suspension resulting from the dilution was put back in the alcohol bath at a temperature of -5°C for 10 minutes (Cazorla et al. 1995). The number of frozen test tubes was counted for each dilution. The total population of INA bacteria/g fresh weight of the sample was estimated by counting the number of frozen tubes in each dilution (Cazorla et al. 1995), then matched to the MPN (Most Probable Number) table according to the Thomas series 333 formula (APHA 1975).

Characterization and identification of INA bacteria

Morphological observation of INA bacterial isolate colonies

Macroscopic morphological observations of the colonies included observations of the shape of the colony, the edge of the colony, the texture of the colony (the condition of the surface of the colony), the size of the colony, and the resulting pigmentation.

Observation of isolates was carried out using Gram stain to determine the type and shape of the cells. Gram staining was carried out by taking a pure culture of INA bacteria aseptically, placing it on a sterile glass object, and then making a suspension with a drop of sterile distilled water. Preparations that had been fixed on a flame were then stained with crystal violet for 1 minute and rinsed with water. Staining was continued with iodine for 2 minutes and rinsed again with water. Bleaching was done with 95% alcohol after rinsing with water, then painted with safranin for 30 seconds. After washing and drying, observations were made with a microscope to see the type of Gram and the shape of the cell (Bangun 1989).

Biochemical test

Catalase test. The INA bacterial isolate that had been fixed on a glass object was added with one drop of 3% H_2O_2 solution, then shaken. The gas formation was observed. The results were positive when bubbles of O_2 gas appeared (Bangun 1989).

Oxidase test. The filter paper was moistened with a 1% *tetramethyl p-phenylenediamine dihydrochloride* reagent. Then the bacterial culture was taken with a sterile straight ose and smeared on the filter paper. When pink, dark red, and black appeared on the paper in the area smeared with the bacterial culture, the test was positive (Hadioetomo 1993).

Indole test. The indole test used the Arnold and Weaver method. It was done by inoculating 2-day-old INA bacteria into the media and then incubating at 37°C. This test used Kovac's reagent, which dripped on the culture after incubation. The reaction was considered positive (+) if a red color was formed on the top layer of the culture. The red color indicated the presence of indole (Cowan 1985).

Data analysis

Observation resulted in quantitative and qualitative data. Qualitative data was morphological and biochemical characteristics of isolates, including observations of color, shape, edges, texture, colony size, Gram stain, catalase test, oxidase test, and indole test, which were analyzed descriptively comparatively by comparing one isolate with other isolates. Quantitative data was the number of INA bacteria analyzed by the Independent-Sample T-Test with a significance of 5% (α= 0.05).

RESULTS AND DISCUSSION

Moss plant sample

Samples of epiphytic and terrestrial mosses were taken from the trekking route of Cemoro Sewu, Mount Lawu, by purposive sampling. Sampling was carried out at three different altitudes, namely 2,026 m asl, 2,331 m asl, and 2,509 m asl, relatively, overgrown with moss. The sample of this moss plant was taken in the rainy season at the end of January 2013. The average results of measurements of abiotic factors at each point of elevation of sampling showed different results with not too much difference in intensity, as shown in Table 1.

Table 1. Abiotic factors at each altitude of moss sampling

Station	Altitude (m asl)	Light intensity (Lux)	Temperature (°C)	Humidity (%)	Soil pH	Soil moisture (%)
1	2,026	589 x 10	21	90	7.5	2
2	2,331	789 x 10	23	77	7	2
3	2,509	652 x 10	22	73	7	1.2

The diversity of abiotic factors is influenced by conditions at different altitudes at the time of sampling. The first station at an altitude of 2,026 m asl and the third at an altitude of 2,509 m asl tend to be wetter due to rain. The second station at an altitude of 2,331 m asl tends to be foggy. Samples of terrestrial mosses and epiphytes from the Cemoro Sewu hiking trail can be seen in Figure 1. Samples of terrestrial mosses growing on rocks are much wetter than epiphytic mosses growing at the base of Pinus merkusii stems. It is because terrestrial mosses are more susceptible to rainwater than more-shaded epiphytic mosses.

Based on observations, the epiphytic mosses mostly grow at the base of the pine trunk. One of the reasons is that there is a lot of humus or soil at the base of the stem so that the rhizoid of the moss is more easily attached to the tree bark. Pine bark as a substrate for epiphytic moss is dry, so the water requirement depends on the rainfall and humidity of the surrounding air. The moss can absorb and retain rainwater, reaching 5-25 times its dry weight. It is the important role of mosses in ecology (Kirmaci and Agcagil 2009).

The comparison results of the morphology of mosses samples showed that epiphytic mosses and terrestrial mosses were of the same species, namely *Campylopus umbellatus* (Arn.) Paris (Leucobryaceae, Musci) (Figure 2) (Sutama 1995; Hasan and Ariyanti 2004; Damayanti 2006; Ignatova and Samkova 2006). Some mosses not only grow epiphytic or terrestrial but can also grow in both places, such as *C. umbellatus*. These results also indicate that this moss was not found in the Mount Lawu area in a previous study conducted by Setyawan and Sugiyarto (2001).

The *C. umbellatus* has several synonyms: *Campylopus coreensis* Cardot., and *Campylopus ferriei* Broth (Jean Edouard Gabriel Narcisse Paris, 1894). This moss is one of the most common species of *Campylopus* found in humid forests. During the rainy season, this moss will grow upright and bright green, while in winter, the color of the leaves of this moss will be black and curved to one side. Talus is like a small tree, 3-5 cm high. Stems grow erect, unbranched, and are covered with fine reddish-brown hairs. The leaves are arranged radially symmetrical, covering the stem. The leaves are needle-shaped with a pointed tip and flat base. The leaf edges are slightly rolled up. Single rib, 1/4-1/3 leaf width, extending to leaf tip. The capsules are brown with short seta (Sutama 1995; Hasan and Ariyanti 2004; Damayanti 2006; Ignatova and Samkova 2006).

Isolation of INA bacteria from moss plants *Campylopus umbellatus*

Isolation of INA bacteria from samples of moss *C. umbellatus* using King's B and NAG media. King's B media is a selective medium for *Pseudomonas* bacteria. It causes *Pseudomonas* colonies to appear fluorescent in King's B media (Arwiyanto et al. 2007). All different colonies found in King's B and NAG media from all samples were tested for ice nucleation activity using a circulating alcohol bath until 7 isolates were positive for INA bacteria, which can be seen in Table 2.

The total number of different isolates obtained from both terrestrial and epiphytic mosses was 76 isolates. All isolates were tested for nucleation activity in a circulating alcohol bath with a temperature of -10°C for 5 minutes, and isolates that were positive for INA bacteria would freeze, as shown in Figure 3.

Colonies that can be categorized as positive for INA bacteria are colonies that have ice nucleation activity. The ice nucleation activity will be seen when the colony suspension in the microtube froze after being put into a circulating alcohol bath with a temperature of -10°C for 5 minutes. The freezing suspension indicates the presence of INA bacteria, which have a single protein as an initiator of ice core formation (Morris et al. 2008). On the other hand, when the suspension of the colony did not freeze, the colony was categorized as negative for INA bacteria.

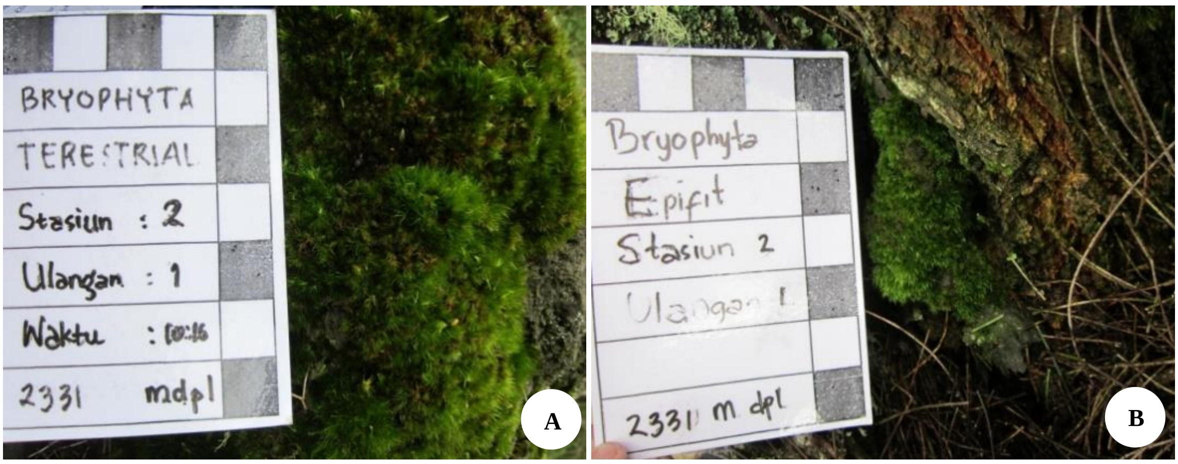

Figure 1. Moss plant samples. A. Terrestrial moss growing on rock surfaces, B. Epiphytic moss growing at the base of *Pinus merkusii* stems

Based on the results above, it can be seen that the most isolates positive for INA bacteria were from station 3, with an altitude of 2,509 m asl in terrestrial mosses. It is because INA bacteria are more commonly found in samples that absorb a lot of rainwater, and terrestrial *C.umbellatus* mosses attached to rocks are more susceptible to rainwater than *C.umbellatus* mosses grow epiphytes on *P.merkusii*. Therefore, considering the distribution of INA bacteria is very closely related to rainwater.

The seven isolates were observed for their morphological characteristics to determine whether they showed different colonies of each isolate, indicating that they were different isolates. The observed colony characteristics included color, shape, edge, elevation, diameter, and structure in the colony, as shown in Table 3 and Figure 4.

Table 3 and Figure 4 show the differences in each isolate's colonies, indicating that the seven isolates are different. For example, isolates 1 and 2 had yellow colony color, circular shape, entire edges, and convex elevation. But the size and structure in the colonies of isolates 1 and 2 were different. Isolate 1 had a colony size of 1500-2000 m, and the internal structure was coarsely granular, while isolate 2 had a colony size of 1000-1500 m and the internal structure was smooth.

Figure 2. Habitus and morphology of moss *Campylopus umbellatus* (40X magnification) A. Habitus of talus of *C. umbellatus*; B. Leaf base; C. Leaf edge; and D. Leaf tip. Description: S: Sporophyte; R1: Flat leaf base; M: Rolled Leaf edge; R2: Pointed leaf tip

Figure 3. Colonies that are positive for INA bacteria: A. Isolate 1, 2, 3; B. Isolate 4, 5, 6; C. Isolate 7; and Control (left to right)

Table 2. Seven isolates that are positive for INA bacteria

Altitude	Number of isolates in terrestrial mosses	Number of isolates in epiphytic mosses	Number of isolates positive INA	Code
2,026	9	12	2 (1 Epiphytic Mosses and 1 Terrestrial Moss)	Isolate 1 (E) Isolate 2 (T)
2,331	10	8	1 (Epiphytic Moss)	Isolate 3 (E)
2,509	17	20	4 (3 Terrestrial Mosses and 1 Epiphytic Moss)	Isolate 4 (T) Isolate 5 (T) Isolate 6 (T) Isolate 7 (E)

Note: T: Terrestrial mosses, and E: Epiphytic mosses

Table 3. Colony morphology isolated from moss plants

Isolate	Color	Shape	Edge	Elevation	Size (μm)	Inner Structure
1	Yellow	*Circular*	*Entire*	*Convex*	1500-2000	*Coarsely granuler*
2	Yellow	*Circular*	*Entire*	*Convex*	1000-1500	*Smooth*
3	White	*Circular*	*Undulate*	*Convex*	1000-1500	*Smooth*
4	White	*Circular*	*Undulate*	*Convex*	1500-2000	*Coarsely granuler*
5	White	*Circular*	*Entire*	*Convex*	1500-2000	*Coarsely granuler*
6	White	*Circular*	*Undulate*	*Convex*	1000-1500	*Smooth*
7	White	*Circular*	*Undulate*	*Convex*	1000-1500	*Smooth*

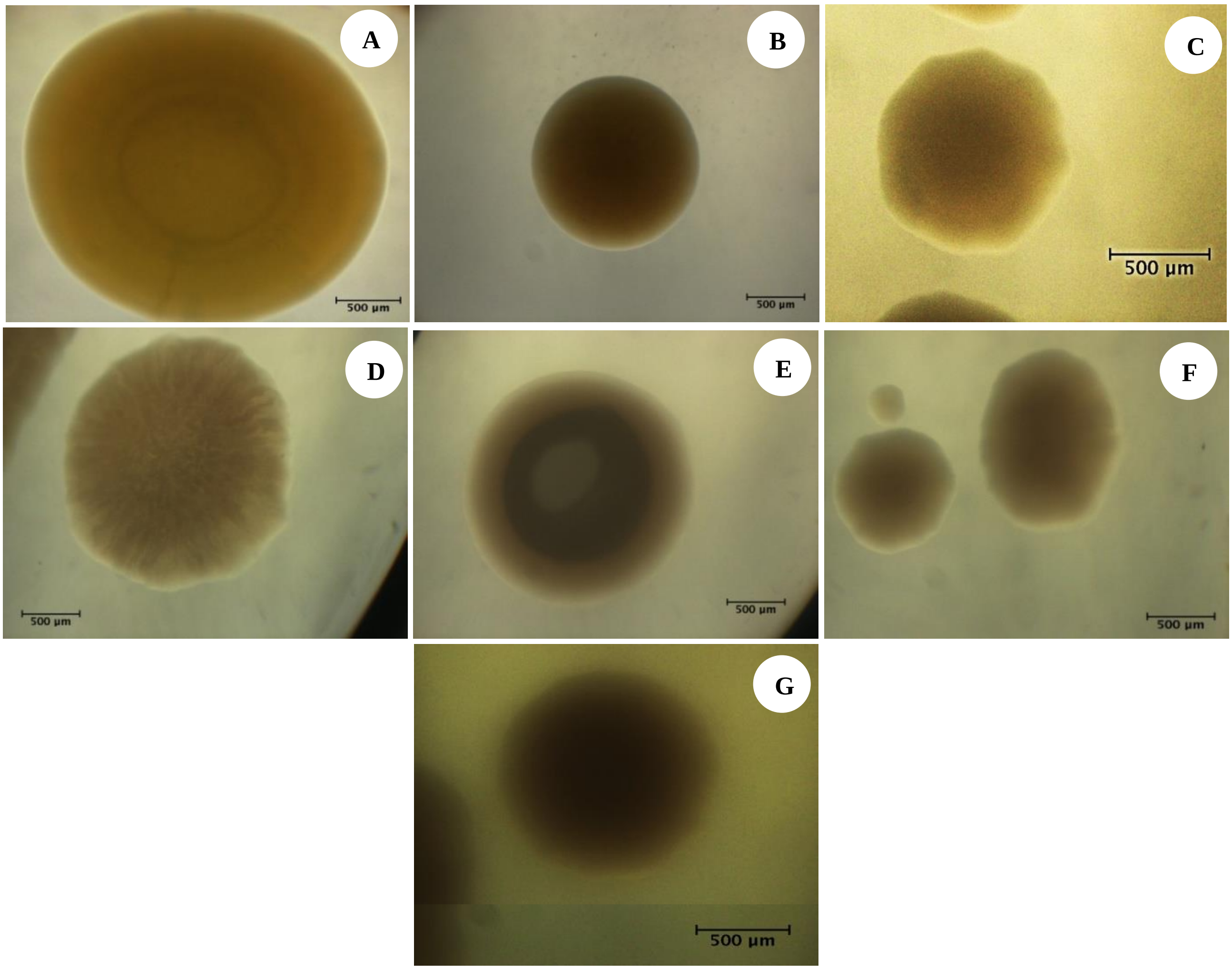

Figure 4. Colony morphology of INA bacterial isolates isolated from moss *C. umbellatus* (40X magnification) A. Isolate 1; B. Isolate 2; C. Isolate 3; D. Isolate 4; E. Isolate 5; F. Isolate 6; G. Isolate 7

Isolates 3, 4, 5, 6, and 7 had white colonies, circular in shape, convex elevation, and all edges were undulate except for isolate 5 with the entire edge. Colony size and internal structure for isolates 3, 6, and 7 were the same, namely 1000-1500 m and smooth, but based on the morphology seen in Figure 4, the three of them looked different. However, colony morphology alone was insufficient to determine the differences between the seven isolates. Therefore, it is necessary to characterize the isolates' cells, including microscopic observations of bacterial isolates and biochemical tests.

Microscopic observation is the most important step in the characterization of bacteria. Microscopic observations of bacterial isolates in the form of cell morphology observations, namely the shape and type of Gram, can be seen from the Gram staining results. Of the seven isolates, all bacteria obtained were rod-shaped. In addition, the Gram staining results on seven bacterial isolates showed that all of them were Gram-negative bacteria which was indicated by the formation of red on the bacterial cells (Figure 5).

Gram-negative bacteria have a cell wall composition mostly composed of a lipid layer, so during staining, they cannot retain the main dye, especially when washed with alcohol, because the lipids are damaged. As a result, this group of bacteria gives a red appearance (the color of the safranin dye) at the end of the Gram staining activity (Hadioetomo 1993).

After microscopic observation of the seven bacterial isolates, it was followed by biochemical tests to determine the physiological properties of bacteria. The biochemical tests included the catalase, oxidase, and indole tests.

The catalase test was carried out to detect the presence of the catalase enzyme, which can convert hydrogen peroxide into water and oxygen. This test is important to determine the nature of bacteria to the need for oxygen. The test results showed that the seven isolates could break down H_2O_2 into H_2O and O_2, as evidenced by the air bubbles on the bacterial preparations after dripping with a 3% H_2O_2 solution. So it can be concluded that the seven isolates were aerobic (Bangun 1989).

The Oxidase test determines the presence of cytochrome oxidase enzymes in certain microorganisms. Cytochromes are respiratory or cellular pigments, which are hemoproteins similar to hemoglobin. These cytochromes are divided into 3 groups, namely cytochromes a, b, and c. Cytochrome c is more abundant in nature than other types of cytochromes. All these cytochromes can undergo oxidation and reduction, and almost all act as hydrogen carriers. This cytochrome is shared by all aerobic bacteria (Salle 1961).

Cytochrome oxidase enzyme is an enzyme that catalyzes the direct transfer of hydrogen by cytochrome c to molecular oxygen and will cause the formation of water. This enzyme is active under aerobic conditions. The results of the oxidase test showed that the seven bacterial isolates produced cytochrome oxidase enzymes. It can be seen in the change in the color of the colony to pink, then dark red, dark red, and finally black (Hadioetomo 1993).

The indole test was used to determine the presence of the tryptophanase enzyme in bacteria. This enzyme can hydrolyze the amino acid tryptophan into indole compounds and pyruvic acid. Indole compounds are spoilage components produced by some bacteria from the amino acid tryptophan. Tryptophan is the only amino acid that naturally contains an indole ring (Salle 1961). The results of the indole test showed that the seven bacterial isolates did not have the tryptophanase enzyme that could hydrolyze the amino acid tryptophan into indole and pyruvic acid compounds. It can be seen from the absence of red color on the agar media after being dropped with Kovacs reagent (Cowan 1985).

The overall characterization results based on morphological and biochemical characteristics in Table 4 show that the seven bacterial isolates could be categorized into 5 different groups. Isolates 3, 6, and 7 were categorized in the same group, while isolates 1, 2, 4, and 5 were in different groups.

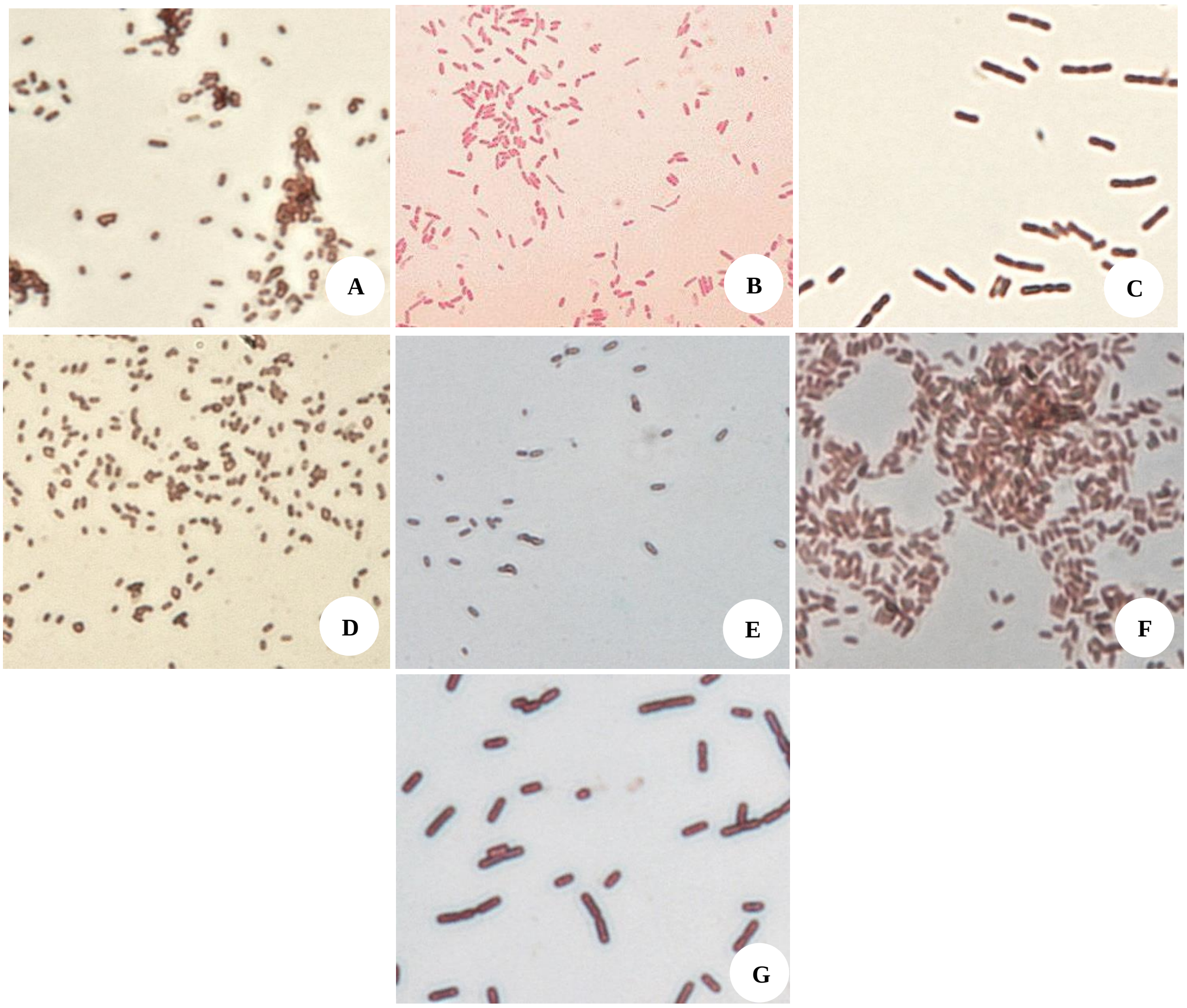

Figure 5. Gram staining of INA bacterial isolates: A. Isolate 1; B. Isolate 2; C. Isolate 3; D. Isolate 4; E. Isolate 5; F. Isolate 6, G. Isolate 7

Table 4. Characterization of 7 Isolates of INA bacteria isolated from moss *C. umbellatus* based on morphological and biochemical characters

Character	Isolate						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Colony color	Yellow	Yellow	White	White	White	White	White
Colony form	*Circular*	*Circular*	*Circular*	*Circular*	*Circular*	*Circular*	*Circular*
Colony edge	*Entire*	*Entire*	*Undulate*	*Undulate*	*Entire*	*Undulate*	*Undulate*
Colony elevation	*Convex*	*Convex*	*Convex*	*Convex*	*Convex*	*Convex*	*Convex*
Colony size (µm)	1500-2000	1000-1500	1000-1500	1500-2000	1500-2000	1000-1500	1000-1500
Inner structure	*Coarsely granuler*	*Smooth*	*Smooth*	*Coarsely granuler*	*Coarsely granuler*	*Smooth*	*Smooth*
Cell shape	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped	Rod-shaped
Gram stain	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+
Indole test	-	-	-	-	-	-	-

Note: + (test result: positive), - (test result: negative)

Table 5. Total population of INA cells/g in epiphytic and terrestrial moss of *C. Umbellatus*

Altitude	Average population of INA	
	Epiphytic moss (cells/g)	**Terrestrial moss (cells/g)**
2,026	50	346
2,331	50	86
2,509	176	396

Estimation of INA bacterial population

INA bacteria are phyllosphere bacteria that grow non-specifically on certain plant species. Its distribution is more influenced by rainwater, humidity, and low air temperature. Therefore, it is necessary to test the estimated population of *C. umbellatus* moss samples to determine the total population at each moss sampling station, terrestrial and epiphytic.

The estimation of the INA population in epiphytic and terrestrial moss samples was obtained using the MPN method of dilution of 3 series of tubes. Each frozen tube in each dilution was counted and matched with the MPN table to obtain the total INA population of cells/g of the moss sample. These results showed that the highest INA population was 930 cells/g, and the lowest was <30 cells/g. The INA population <30 cells/g was not sufficient to freeze 10 ml of phosphate buffer, indicated by the absence of a frozen tube in each dilution.

Based on the results of MPN in each replication, the average population of INA bacteria cells/g of epiphytic and terrestrial *C. umbellatus* mosses at each station was calculated and presented in Table 5.

The data shows the population of INA (cells/g) of epiphytic moss *C. umbellatus* growing at stations 1 and 2 is the same, namely 50 cells/g, and the highest is at station 3, 176 cells/g. While the number of INA population (cells/g) of terrestrial moss *C. umbellatus* was lowest at station 2, namely 86 cells/g, and the highest was at station 3, namely 396 cells/g. The diversity of these results is influenced by abiotic factors around the habitat environment, which vary from one habitat to another. In addition, INA bacteria do not have a specific leaf habitat. Therefore, the activity and number of INA bacteria populations in several locations are more influenced by the season and the amount of rainfall (Morris et al. 2004).

Based on the results of the Independent-Sample T-Test, the comparison of the results of the INA bacterial population between terrestrial and epiphytic *C. umbellatus* mosses showed the number 0.017 in its significance value (Appendix 2). It means that since the significance value ≤ 0.05, then Ho is rejected. The average INA (cells/g) population of terrestrial mosses is greater than that of epiphytic mosses. The average population of INA bacteria in terrestrial mosses, which was 276 ± 377.9 cells/g, was greater than that of epiphytic mosses, which was only 92 ± 129.4 cells/g. It was because terrestrial mosses which lived in open areas received more rainwater than epiphytic mosses which lived in more shaded areas.

Previous research stated that INA bacteria are taken from rainwater, and air between March-May 2008 from Jakarta, Bogor, Bekasi, Tangerang, and Depok obtained the percentage of INA population in each area of 19.4%, 18.7%, 5, 3%, 2.2%, and 6.4% and 9.5%, 6.5%, 0%, 2.7%, and 1.8%, respectively. It shows that the percentage of the population of INA bacteria in samples from rainwater in all these areas is greater than that from the air. The presence of INA bacteria in rainwater and air may play an important role in the nucleation process required for rain induction (Stephanie and Waturangi 2011).

From this research, it can be concluded as follows: (i) 7 isolates of INA bacteria were isolated from the moss *C. umbellatus* on the trekking route of Cemoro Sewu, Mount Lawu; (ii) the average number of INA bacteria in the moss *C. umbellatus* terrestrial mosses, namely 346 cells/g, 86 cells/g, and 396 cells/g, were larger than the moss *C. umbellatus* epiphytes, namely 50 cells/g, 50 cells/g, and 176 cells/g, at stations 1, 2, and 3 on the trekking route of Cemoro Sewu, Mount Lawu.

REFRENCES

APHA. 1975. Standard Methods for the Examination of Water and Wastewater, 14th Edition. American Public Health Association. Washington, DC.

Arwiyanto T, Maryudani YMS, Azizah NN. 2007. Sifat - sifat fenotipik *Pseudomonas fluoresen*, agensia pengendalian hayati penyakit lincat pada tembakau Temanggung. Biodiversitas 8 (2): 147-151. DOI: 10.13057/biodiv/d080215. [Indonesian]

Bangun A. 1989. Isolasi dan Identifikasi Bakteri. PAU Bioteknologi Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Cazorla FM., Olalla L, Tores JA, Perez-Garcia A, Codina JC, de Vicente A. 1995. A method for estimation of population densities of Ice Nucleation Active *Pseudomonas syringae* In Buds and Leaves of Mango. J Appl Bacteriol 79: 341-346. DOI: 10.1111/j.1365-2672.1995.tb03146.x.

Christner BC, Cai R, Morris CE, McCarter KS, Foreman CM, Skidmore ML, Montross SN, Sands DC. 2008b. Geographic, seasonal, and presipitation chemistry influence on the abudance and activity of biological Ice Nucleators in rain and snow. Proc Natl Acad Sci USA 105 (48): 1-6. DOI: 10.1073/pnas.0809816105.

Christner BC, Morris CE, Foreman CM, Cai R, Sands DC. 2008a. Ubiquity of biological Ice Nucleators in snowfall. Science 319: 1214-1217. DOI: 10.1126/science.1149757.

Cowan ST. 1985. Manual for the Identification of Medical Bacteria. Cambridge University Press, London.

Damayanti, L. 2006. Koleksi Bryophyta Taman Lumut Kebun Raya Cibodas. UPT Balai Konservasi Tumbuhan Kebun Raya Cibodas, Cianjur. [Indonesian]

Edwards AR, Ronald A, Wichman HA, Orser CS. 1994. Unusual pattern of bacterial Ice Nucleation gene evolution. Mol Biol Evol 11: 911-920. DOI: 10.1093/oxfordjournals.molbev.a040172.

Gurian-Sherman D, Lindow SE. 1993. Bacterial Ice Nucleation: Significance and molecular basis. Proc Fla State Hort Soc J 7:1338-1343. DOI: 10.1096/fasebj.7.14.8224607.

Hadioetomo RS. 1993. Mikrobiologi Dasar Dalam Praktek. Gramedia Pustaka Utama, Jakarta. [Indonesian]

Hasan M, Ariyanti NS. 2004. Mengenal Bryophyta (Lumut) Taman Nasional Gunung Gede Pangrango. Volume 1. Balai Taman Nasional Gunung Gede Pangrango, Cibodas. [Indonesian]

Ignatova EA, Samkova TY. 2006. *Campylopus umbellatus* (Arn.) Paris (Leucobryaceae, Musci)- A new spesies For Russia. Arctoa 15: 215-218. DOI: 10.15298/arctoa.15.11.

Kartika A. 2009. Teknik Eksplorasi dan Pengembangan Bakteri Pseudomonas flourescens. Laboratorium Pengamatan Hama dan Penyakit (PHP), Banyumas. [Indonesian]

King EO, Ward, MK, Raney ED. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med 44: 301-307.

Kirmaci M, Agcagil E. 2009. The Bryophyte flora in the urban area of Aydin (Turkey). Int J Botany Stud 5 (3): 216-225. DOI: 10.3923/ijb.2009.216.225.

Lindow SE, Arny DC, Upper CD. 1978. Distribution of Ice Nucleat- ion-Active bacteria on plants in nature. Appl Environ Microbiol 36: 1-838. DOI: 10.1128/aem.36.6.831-838.1978.

Lindow SE, Arny DC, Upper CD. 1982. Bacterial Ice Nucleation: A Factor in Frost Injury to Plant. Plant Physiol. 70:1084-1089. DOI: 10.1104/pp.70.4.1084.

Morris CE, Gergakopoulos DG, Sands DC. 2004. Ice Nucleation Active bacteria and their potential role in precipitation. J Phys IV France 121: 87-103. DOI: 10.1051/jp4:2004121004.

Morris CE, Sands DC, Bardin M, Jaenicke R, Vogel B, Leyronas C, Ariya PA, Psenner R. 2008. Microbiology and atmospheric processes: An upcoming era of research on bio-meteorology. Biogeosci Discuss 5: 191-212. DOI: 10.5194/bgd-5-191-2008.

Salle AJ. 1961. Fundamental Principles of Bacteriology. McGraw Hill Book Co. Inc., New York.

Setyawan AD, Sugiyarto. 2001. Keanekaragaman flora Hutan Jobolarangan Gunung Lawu: 1. Cryptogamae. Biodiversitas 2 (1): 115-122. [Indonesian]

Stephanie, Waturangi DE. 2011. Distribution of Ice Nucleation Active (INA) bacteria from rain-water and air. Hayati 18 (3):108-112. DOI: 10.4308/hjb.18.3.108.

Sutama. 1995. Keanekaragaman Jenis Lumut di Sekitar Kawah Dataran Tinggi Dieng. [Skripsi]. Fakultas Biologi Universitas Gadjah Mada, Yogyakarta. [Indonesian]

Wahyudi AT. 1995. Pembentukan inti es oleh bakteri. Hayati 2:55-59. [Indonesian]

Waturangi DE, Amelia T. 2009. Isolation, characterization and genetic diversity of Ice Nucleation Active bacteria on various plants. Hayati 16 (2): 54-58. DOI: 10.4308/hjb.16.2.54.

Windadri FI. 2007. Lumut (Musci) di Kawasan Cagar Alam Kakenauwe dan Suaka Margasatwa Lambusango, Pulau Buton, Sulawesi Tenggara. Biodiversitas 8 (3): 197-203. DOI: 10.13057/biodiv/d080307. [Indonesian]