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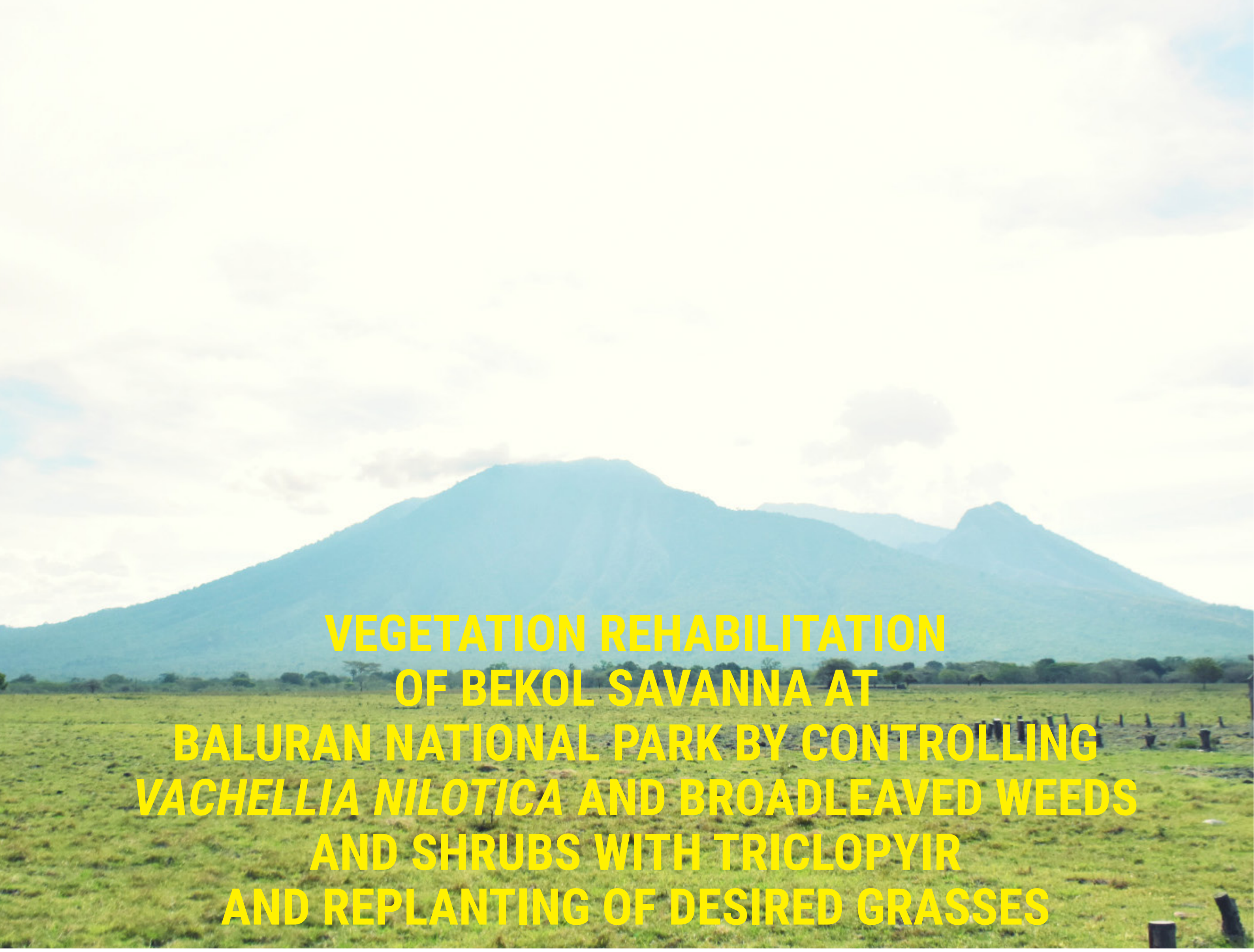

VEGETATION REHABILITATION OF BEKOL SAVANNA AT BALURAN NATIONAL PARK BY CONTROLLING *VACHELLIA NILOTICA* AND BROADLEAVED WEEDS AND SHRUBS WITH TRICLOPYIR AND REPLANTING OF DESIRED GRASSES

Soekisman Tjitrosemito[1*], Ragil Irianto[2], Garsetiarsih[2], Titiek Setyawati[2], Atok Subiyakto[2], Adi Susmianto[2]

[1]Affiliate Scientist, SEAMEO BIOTROP. Jalan Raya Tajur Km 6, Bogor

[2]Researcher at the Indonesia Forest Research and Innovation Center, Jln. Gunung Batu 5, Bogor

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***Corresponding author:**
s.tjitrosemito@biotrop.org

ABSTRACT

Research works were carried out from 2012 – 2015 to investigate the vegetation rehabilitation by controlling broadleaved shrubs, shrubs and trees of *Vachellia nilotica* (the reviewed name of *Acacia nilotica*) and planting desired grasses The treatments combined 3 factors. Factor 1, was the way to apply triclopyr formulated as GARLON 670 EC to control trees of *V. nilotica*, (1) by brushing the solution of 1% GARLON 670 EC dissolved in diesel oil on the stump of *V. nilotica* after cutting using a chainsaw, and by (2) Brushing 1% solution of GARLON 670 EC at the base of intact standing tree of *V. nilotica*, from above the ground up to 30 cm height. Factor 2, was grasses (1) *Dichanthium caricosum*, and (2) *Polytrias amaura* planted ad 1 x 1 m^2. Factor 3 was fertilization on, (1) planted grasses were fertilized with compost derived from goat feces at 100 gr/ single chunk planted, and (2) without fertilizer. The results indicated that grass grew better under the treatment of killing *V. nilotica* by cutting and brushing with 1% GARLON 670 EC dissolved in diesel oil, while fertilizing *D. caricosum* was much better to than of *P. amaura*.

INTRODUCTION

The ecosystems of Bekol savanna and other savanna in Baluran National Park rest on a long dry season with a limited water supply during the wet season; this monsoonal area supports the growth of grasses with small trees here and there to form savanna ecosystems. The ecosystem constitutes its foodweb with its main herbivores are banteng (*Bos javanicus*), beside deers, buffaloes, jungle fowl and peacocks, with their carnivors of wild dog and previously also extinc javan tiger. During the colonial time the area was kept as a prestine ecosystem; in the wetter surrounding area they established teak forest plantation with a high quality teak timber production. After independence the teak forest plantation was managed by PERHUTANI, the estate forest plantation. Savannas in Baluran National Park (especially Bekol savanna) undergoes regular fire annually. The management of PERHUTANI, looking at a huge teak leaves litter deposited in the teak forest floor, worried that those biomass fuel will be ignited by the enchroaching savanna fires. It may create a fire disaster in the teak forest plantation. Apprehasive about the possibility of disaster, in 1969 the manager of nearby PERHUTANI decided to plant *V. nilotica* as a fire break about 2 km along the fench separating the teak forest plantation from Bekol savanna (Alikodra,1987). During this writing there was a heated discussion about the origin of the *V. nilotica* seeds planted along that fench. Some workers claimed that they were involved in planting of *V. nilotica* and admited they picked up pods from neaby areas. However they did not know who brought the original *V. nilotica* seed to the areas.

V. nilotica (syn. *Acacia arabica*), was imported from India in the 19th century studied in Bogor Botanical Garden for a possibility of producing "gum" (arabic acid) a product which was valued expensively at that time. It was common in the world to market *Acacia* gums collected from many kinds of *Acacia* , therefore, the quality varied considerably. The colonial botanists must have expected that *A. arabica* would produce a good quantity of arabic gum. The research indicated that *A. arabica* grew well, but produced only a small amount of low quality gum, and the experimental works were soon terminated. The colonial botanists must have thought that *V. nilotica* was a good plant and never thought of invasive whatsoever, and some of the seeds were sent to Palu (Central Sulawesi) some to Bali and some to Timor. Later on it was shown in the literature that *Acacia* producing a good quality of arabic gum was *Acacia senegalensis* not *V. arabica* (syn. *Acacia nilotica*).

The *V. nilotica* fench as a fire brake worked well, preventing fire from enchroaching the teak forest plantation, and it found suitable environment, plenty of sunshine supporting its rapid growth and proliferation. It also enjoyes a symbiotic relationship with local herbivores to spread its seeds all over the place in the park to become terribly invasive. During the dry season when soil moisture went down really low, and air temperature soared high almost all vegetation were dried out, and those herbivores suffered from lack of herbage and under full stress of thirsty. In this condition riped, matured pods of *V. nilotica* were shed, and dropped on the savanna floor. The wondering hungered and thirsty herbivores found those pods palatable and they eat gregariously to quench their hunger and thirst. Those pods turned out to be nutricious, containing high fat and protein. The pods were digested well, however most the the seeds were undamaged after going through herbivores digestive tract, and were excreted intact in the feces of those herbivores. The seeds even enjoyed a good moist and fertile growing media supporting their successful germination. This perfect symbiotic relationship indeed supports a rapid distribution of *V. nilotica* in the park, forming a dense canopy of *V. nilotica*, shading the grasses out.

In 1980's a little bit more than 10 years after fench planting, the rapid expansion of *V. nilotica* drew an attention of the park manager to control it. The approach was to cut the stem manually and burnt the plant biomass. The stum, instead of dying, coppiced profously changing the single stem into multistem *V. nilotica* plant creating a thick canopy preventing a greater proportion of light penetration. The reduction of light penetration reduced the growth of *Dichantium caricosum*, a highly preffered grass by herbivors, and competed out by *Brachiaria reptans, Sclerachne punctata* in some areas also Oplimenus compositu, shade tolerant grasses. The situation become worse, because this low light intensity also preferred broadleaved weeds to come (Germer, 2003), such as *Achiranthes aspera, Bidens biternata Hyptis suaveolens, Eleutherantera ruderalis, Flemengia lineata, Ocimum canum, Thespesia lampas, Vernonea cymosa,* etc. It seemed the approach was inappropriate. Chemical control using 2,4-D, triclopyr and glyphosate were also tried unsuccessfully, due to a limited knowledge on herbicidal mode of actions and applications. The mechanical control using bulldozer to uproot *V. nilotica* proved to be successful. About 125 ha savanna in the Bekol was cleared of standing *V. nilotica* trees, and become a site for visitors to watch inhabiting fauna sepecially banteng. However it could not recover *D. caricosum*, and worse the cleared savanna was now dominated by those broadleaved weeds especially *T. lampas, V. cymosa*, and *F. lineata*.

Those above descriptions were a rough picture of Savanna in Baluran National Park. A recent vegetation map (Setiabudi et al., 2013) indicated that there were at least 7 different degree of *V. nilotica* invasions from a full *V. nilotica* canopy coverage to sparse *V. nilotica* distribution, covering a total area of more than 6000 ha. Under those different degree of invasions there were a wide variation of vegetation composition, from only small grasses and other vegetation, followed by a dense herbs or shrubs with a thin shade tolerant grasses, and to sparse *V. nilotica* distribution, with a perrenial grass *D. caricosum* was still dominant producing excellent herbage. So there were at least 3 problems that must be overcome to rehabilitate those savanna: (1) to eradicate or control *V. nilotica*, (2) To eradicate or control herbs and shrubs to facilitate the recovery of grasses, and (3) to replant grasses where they were absent or at much lower composition than herbs and shrubs. This experiment was aimed at rehabilitating savanna vegetation at Bekol.

Recent Fidings

From the recent vegetation map developed by Setiabudi et al. (2013), a greater part of *V. nilotica* invasions was followed by the growth of herbs and shrubs underneath. A smaller portion of *V. nilotica* invasion with full canopy of *V. nilotica* created almost bare without vegetation was inadvertently induced by unsuccessful control of *V. nilotica* which allowed the stump to sprout 6-13 buds to grow into new stems. To prevent the stump from sprouting it must be killed and this can be done by applying tryclopyr (formulated as GARLON 670 EC) disolved in diesel oil. An experiment conducted in June and evaluated in November 2012 on *V. nilotica* trees that were cut and the stumps were immediately brushed with Garlon 670 EC diluted in diesel oil using a soft paint brush showed successfuly killed *V. nilotica* (Table 1).

Table 1 showed that even control, without any herbicide treatment reduced sprouting almost 40%. This was due to inconsistency of plants in the field during dry season, as it was noticed that some trees with cut stump under no herbicide treatment was found also dead, it was simply due to the soil environmental variability as the condition was so dry. The valuable results of the experiment were indicated by these data that herbicide concentration from 1.5 – 12 ml triclopyr in 100 ml diesel oil reduced the sprouting ability of cut stump of *V. nilotica* by more than 80%, in other word the concentraion of triclopyr as Garlon at 1.5 ml/100 ml diesel oil or about 1.5% (by volume) was sufficient to kill *V. nilotica* trees . A higher concentration up to 12 ml/100 ml gave a better result almost killed the tree but still leaving 7% to be retreated again.

The impact of different stump height on the survival of *V. nilotica* stump was shown in Table 2 indicating that the stump height of 10 cm was the best in reducing the ability to survive by almost 90%. It is realised that brushing 10 cm height stump with herbicides is a back breaking jobs, therefore, the supervisor must be able take care and ensure that the herbicide solution is delivered correctly and consistently, attended from time to time to ensure that stumps are treated immediately after cutting.

When *V. nilotica* was successfuly controlled herbs and shrubs, the second problem still dominated the vegetation and competed grasses out. The following was the composition of vegetation under *V. nilotica* with medium canopy coverage measured in term of SDR (Summed Dominance Ratio). See Table 3.

Table 3 indicated that the vegetation composition was dominated by herbs and shrubs such as *E. ruderalis*, *H. suaveolens*, *Bidens biternata*, *Achiranthes aspera*, *Aeschynomene indica*, even also climbing *Ipomoea alba*, leaving only less than 25% grasses *Oplismenus compositus* and *B. reptans* that were shade tolerant but less palatable to herbivores. Although the vegetation composition varied greatly under the variable *V. nilotica* canopy, a considerable proportion herbs and shrubs was common. It was important therefore to control them to allow grasses to recover or to replant selected grasses. An experiment conducted during wet season of February 2013 application of triclopyr (formulated as GARLON 670 EC) at 1.0 lt/ha sprayed using knapsack sprayer in 400 lt water, 0.2% Agristick using T-jet nozzle calibrated at High Pressure successfuly controlled those herbage and shrubs (see Table 4).

Table 1. The sprouting percentage of *V. nilotica* stump after triclopyr applications (Tjitrosoedirdjo et al., 2013)

	Triclopyr application (g/lt)				
	0	**1.5**	**3**	**6**	**12**
% stum sprouting	63.1[a]	19.4[b]	15.5[b]	14.0[b]	6.6[c]

NB. Numbers followed by the same letter did not differ significantly at 5%

Table 2. The sprouting percentage of *V. nilotica* stum after triclopyr applications (Tjitrosoedirdjo et al., 2013)

	Treatments (stump height)		
	10 cm	**15 cm**	**30cm**
% stump sprouting	11[a]	27[b]	35[c]

NB. Numbers in one column followed by the same letter did not differ significantly at 5%

Table 3. Vegetation composition in the Bekol savanna during the wet season of February 2013 under the medium canopy of *V. nilotica* (Tjitrosoedirdjo et al., 2013).

No	Species	Family	SDR	No	Species	Family	SDR
1	*Achiranthes aspera*	Amarantaceae	2.86	13	*Ipomoea alba*	Convolvulaceae	6.29
2	*Aeschynomene indica*	Fabaceae	3.65	14	*Ipomoea sp.*	Convolvulaceae	2.92
3	*Bidens biternata*	Asteraceae	9.11	15	*Merremia emarginata*	Convolvulaceae	0.52
4	*Brachiaria reptans*	Poaceae	3.59	16	*Mimosa diplotrica*	Fabaceae	1.04
5	*Ceyrasia tripolia*	Vitaceae	0.69	17	*Ocimum canum*	Lamiaceae	0.57
6	*Centrosema sp.*	Fabaceae	0.56	18	*Oplismenus compositus*	Poaceae	20.9
7	*Cleome gynandra*	Capparidaceae	1.60	19	*Phyllanthus debilis*	Euphorbiaceae	0.45
8	*Commelina sp.*	Commelinaceae	0.47	20	*Phyllanthus niruri*	Euphorbiaceae	1.95
9	*Corchorus clitorius*	Tiliaceae	2.10	21	*S'dling V. nilotica*	Fabaceae	0.53
10	*Digera arvensis*	Amaranthaceae	0.45	22	*S'dling A. indica*	Meliaceae	1.38
11	*Eleutherantera ruderalis*	Asteraceae	26.0	23	*Thespesia lampas*	Malvaceae	0.95
12	*Hyptis suaveolens*	Lamiaceae	11.6	24	*Vernonia cymosa*	Asreraceae	0.55
			Total				**100**

Table 4. The efficacy of foliar spray of some selective herbicides for herbs and shrubs applied during the wet season of February 2013. (Tjitrosoedirdjo et al., 2013)

N0	Treatments	Impact on herbs and shrubs and grasses	
		Percent coverage of herbs and shrubs	**Percent Coverage of Grasses**
1	Fluroxypir, 0,75 l/ha	10.58[b]	83.23[a]
2	Triclopyr, 0.5 l/ha	21.79[b]	62.58[b]
3	Triclopyr 1.0 l/ha	7.66[b]	80.52[a]
4	2,4-D 1.0 l/ha	12.71[b]	78.13[ab]
5	2,4-D 2.0 l/ha	8.69[b]	80.52[a]
6	Control	58.79[a]	32.48[c]

NB. Numbers in a column followed by the same letter did not differ significantly at 5%

MATERIALS AND METHODS

With the above information an experiment under the FORIS project was designed to follow up further in overcomming the problems of rehabilitating vegetation in savanna of Baluran National Park. The general way of killing *V. nilotica* and controlling the dominating herbs and shrubs were known, i.e. by using trilopyr (formulated as GARLON 670 EC) at 1.5-12.0 ml/100 ml of diesel oil brushed using soft painting brush on stump immediately after cutting. While those herbs and shrubs growing in between grasses can be selectively controlled using fluroxypir (STARENE), 2,4-D (LINDOMIN), mainly triclopyr formulated as GARLON 670 EC at 1.0 lt/ha sprayed at 400 lt water mixed with Agristick as surfactan at 0.2 % solution.

The experimental design was factorials with 3 factors, first factor was the methods of applying triclopyr, (1) by brushing 1% GALON 670 EC, diluted in 100 ml diesel oil on stump of *V. nilotica* after cutting, and (2) brushing 1% GALON 670 EC, diluted in 100 ml diesel oil at the base of standing *V. nilotica* tree from the ground surface up to about 30 cm ; the second factor was planting palatable grasses, i.e. (1) *D. caricosum* and (2) *P. amaura*: and the third factor was fertilizer using compost derived from feces of goat, i.e. (1) with compost and (2) without compost. The experimental treatments were a combination of 23 factors giving a total of 8 treatments and replicated 4 times. There were 32 plots each measuring 7 x 7 m^2. The treatments were randomized going through the first factor, followed by the second and third factor. After completing the designated treatments, all the 32 plots were sprayed with 1 lt GARLON 670 EC/ha using knapsack sprayer calibrated to deliver 400 lt of water/ha, added with 0.2% Agristick as a surfantant using T-jet nozzle and High pressure. (This spraying is not an experimental treatment, but only to adopt the previous good results that was able to reduce the coverage of shrubs and broadleaved weeds). Three days after spraying, grass treatments (*D. carisoum* and *P. amaura*) were applied and planted spaced at 1 x 1 m^2, using pieces of grass sod. The following day, the fertilizer treatments were applied as top dressing of a bucket of mature compost (about 100 gr) at designated plots. The last applied treatments were cutting trees of *V. nilotica* followed by brushing 1% GARLON 670 dissolved in diesel oil on cut stump, and brushing 1% GARLON 670 on standing trees from the ground surface up to 30 cm height. Those plots were fenched with 4 lines of barb wire nailed on 2 m poles of *V. nilotica* trees spaced at 10 m away around the plots to prevent a possibe disturbance from animals. The experimental lay out was displayed in Figure 1.

The selected area was slightly undulating, representing a typical area under the shade of *V. nilotica* invasion of medium canopy in Bekol savanna. Under the shade of *V. nilotica* was dominated by herbs and shrubs, although some areas were noticably bare (outside the treatment areas). Tree months after treatment applications the surviving *V. nilotica* trees (indicated by resprouting from standing trees or stumps) were counted, as variables to measure the efficacy of triclopyr applications The vegetation compositions under *V. nilotica* of each treatments were sampled using quadrate measuring 1 x 1 m^2, repeated 5 times in a non destructive method. All the species and their densities were recorded. The data on species composition in each treatments were subjected to cluster analysis. The coverage of grasses were measured in each samples and differentiated from that of herbs and shrubs and utilized to measure grass the recovery

This cluster analysis is based upon the distance between two vegetation compositions that are generated the following way:

d (i, j) = 1- IS, where d (i,j) is the distance between treatment i and j for i,j = 1,2.....8.

While IS is an Index of similarity of Czekanowski (1913) :

$$\frac{2 \sum \min (x_i, y_i)}{\sum (x_i + y_i)}$$

Where x_i and yi are a number of species i.

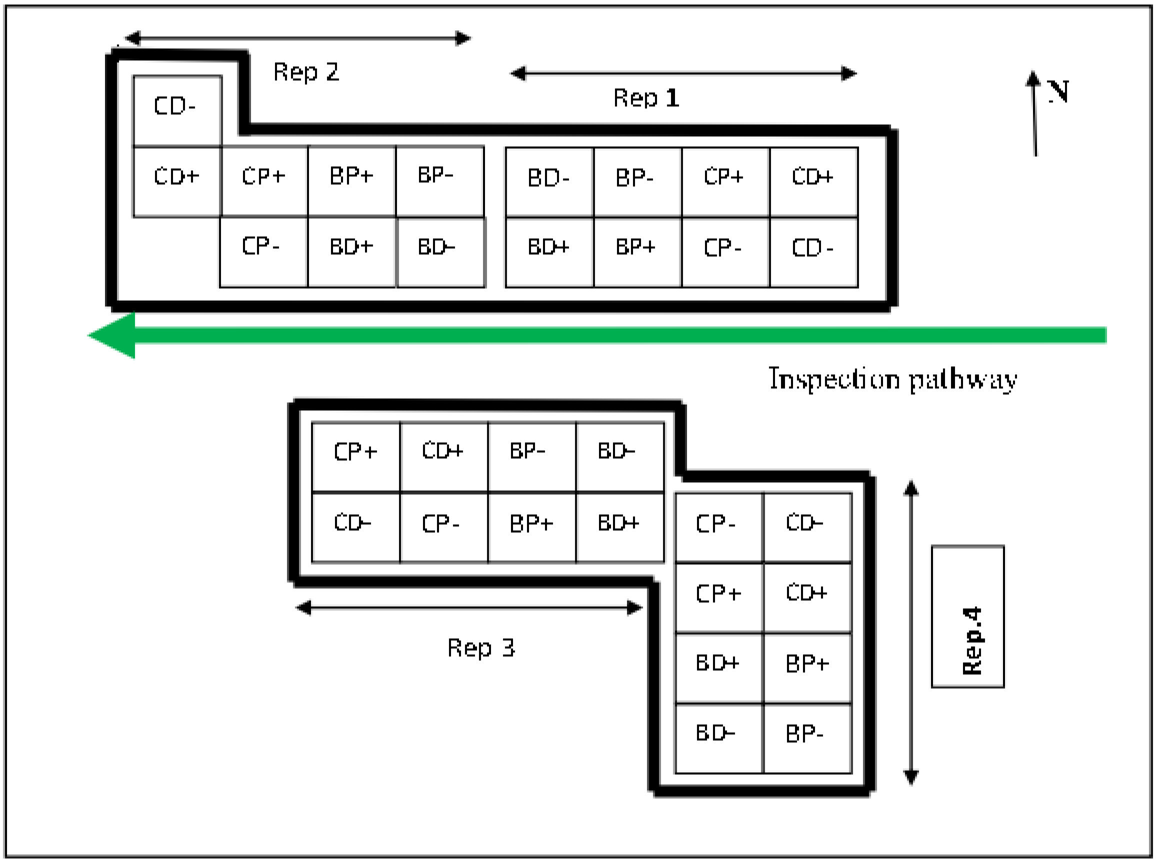

Figure 1. Experimental layout of treatments in the field; **factor I,** Control of *V. nilotica* trees, **C** = cut the tree and the stump brushed with triclopyr at 1.5 ml/l00 ml dissolved in diesel oil; **B** = brushed at the base of standing *V. nilotica* trees with triclopyr at 1.5 ml/l00 ml dissolved in diesel oil; **factor II**, planted grasses, **D** = *Dicanthium caricosum*, **P** = *Polytrias amaura*; **factor III**, + = with a bucket (100 g) of compost, - = without a bucket (100 g) of compost.

This cluster analysis is based on a simple minimum distance or the nearest neighbor method (McGarigal et al., 2000). The main input in the algorithm for this minimum distance is the matrix of distances between treatments. The algorithm of the minimum distance method is solved as follows:

1. In this experiment, there are 8 treatments, represented by 8 different vegetation compositions (species); there will be a square matrix of 8 x 8 dimensions with IS between treatments as its member. The IS matrix above must be changed into a matrix of distance D = d {i, j} with 8 x 8 dimensions.
2. Select the smallest distance pair to be combined from the D matrix. For example, the smallest distance is d {x, y}, which means that the treatments or vegetation composition x and y are the most likely to be combined because they have similar composition.
3. As the minimum distance is d = {x, y} so vegetation compositions of x and y may be combined into a new vegetation composition and named the vegetation composition of (x, y). After combination, it is important to adjust the D matrix in the following way:
   a. Delete column and row related to vegetation composition of x and y,
   b. Add a row and column containing the value of vegetation composition distance (x, y) and the remaining vegetation compositions.
   c. The distance value among community pairs (x, y) and z is determined by following this formula: d {(x,y), z } = min {d (x, z), d (y, z)}.
   d. Steps b and c are repeated seven times until all the vegetation compositions are combined into one.

It is important to record the vegetation composition identity and the minimum distance when combined.

RESULTS AND DISCUSSIONS

The evaluation carried out 3 months after the application of triclopyr as 1% GARLON 670 EC diluted in diesel oil brushed on the stump, no regrowth was recorded from the stump. However, under the treatment of brushing at the base of standing trees, some of treated trees, although leaves were shed, branches started to resprout, trees were not killed. It seemed the concentration was not enough. The surviving trees were re treated again with the same concentration to kill them all.

The species composition under *V. nilotica* canopy 3 months after treatments were presented in Table 5. These vegetation (species) compositions were the result of foliar application of triclopyr as 1 lt GARLON 670 EC/ha was promising. The growth of herbs and shrubs were reduced leaving only *Bidens biternata, Eleutheranthera ruderalis, Vernonea cymosa*. These herbs are members of Asteraceae capable of producing small capsellas with pappus which are easily blown by the wind. However they are quite susceptible to selective herbicides such as triclopyr, fluroxypir or 2,4-D. Other shrubs such as *Jatropha gossypiifolia, Thespesia lampas, Ocimum canum, Achyranthes aspera, Hyptis suaveolens* are more difficult to control, also other climbing vines such as *Merremia emarginata, Calopogonium mucumoides*, especially *Mucuna*, and *Flemengia lineata* the noxious stoloniferous herb. The rate of triclopyr as 1lt GARLON EC/ha may be too low, 2 lt GARLON 670EC/ha will be better in killing a greater part of those herbs and shrubs, therefore providing opportunity for grasses to recover. The growth of grasses becoming dominant, especially *B. reptans*.

The above experimental results will help to solve the problems of killing those nuisance standing herbs and shrubs, but still leaving a considerable number of seed in the ground. However if the control of these herbs and shrubs are consistantly carried out before they produce viable seeds, in four years most of those herbs and shrubs would have considerably been reduced, at the same time grasses would again dominate the savanna. These herbs and shrubs dry out during dry season (June – November) at the peak of dry season some of the areas are burnt, almost all those herbs and shrubs would have died away. However at the beginning of wet season seeds of herbs and shrubs will germinate and grow prolifically competing grasses out. At approximately January/February (depending upon the outset of rainy seson) these herbs and shrubs should be sprayed with triclopyr or fluroxypir or 2,4-D to kill those herbs and shrubs.

The results of cluster analysis of those 8 different species compositions were presented in Figure 2. It was easily noticed that the vegetation compositions in all 8 different treatments were dominated by *B. reptans*. This dendrogram provides a very interesting results. When the line of 50% similarity is taken as the criteria to differentiate the vegetation compositions, i.e., if IS < 50% those vegetation compositions differ one from the other; if IS>50% the 2 compared species compositions do not differ one from the other. Under this criteria the whole 8 communities were similar, because the value of IS combining all vegetarion composition was less than 50%. It was interesting, the foliar application of triclopyr at 670 g.ai/ha on those herbs and shrubs during the wet season was effective in controlling them, and provided an opportunity for grasses to recover (greater coverage). However if we utilize a line of 80% of similarity simply to seperate the species compositions, those 8 communities are split into 2, i.e. vegetation type I consisting of composisition CD-, CP+, CD+, CP- and BD+ representing the application of 1% GARLON 670 EC on stump while type II consisting of composition BP+, BD- and BP- representing the application of 1% GARLON 670 EC at the base of standing tree.

The impact of *V. nilotica* control using different method of 1% GARLON 670 EC application, not only directly impacting on the regrowth of *V. nilotica* but also affected the recovery of grasses as shown in Table 6. The treatment application of 1% GARLON 670 EC on stump, induced the grass recovery greater ($P \leq 0.05$) than the application at the base of standing tree.

The cuting of *V. nilotica* trees followed killing the stumps by applying 1%GARLON 670 EC directly on the stump removed the canopy shade, increased the availability of sun light for grasses to photosynthesize. By removing *V. nilotica* trees also reduced the evaporative demand of soil water, therefore the availability of soil water was better. These conditions supported a good growth of grasses. While the application of 1% GARLON 670 EC at the base of *V. nilotica* tree did not kill those trees immediately, presumably water traspiration stayed high reducing the soil moisture and reducing sun light for photosynthesis for grasses Table 6. The grass coverage as effected by the application of GARLON 670 EC on *V. nilotica*

However the biomass of planted *D. caricosum* and *P. amaura* 4 months after planting and fertilization presented a more complicated data. See Table 7.

There was an indication that biomass of *D. caricosum* (L). A. Camus during the 4 months growth was higher than that of *P. amaura* ($P \leq 0.01$). However the way to apply GARLON 670 EC to kill *V. nilotica* wether directly brushed at the base of the tree or on stump, as well as the fertilization treatments did not effect the *D. caricosum* nor *P. amaura* growth. The experimental area contributed to the high variability of the growth of planted grasses. *D. caricosum* is a creeping stoloniferous perennial with blue-tinged stems and fine pointed leaves, 4–20 cm long, 2–6 mm wide. Stolons can grow to 1.5–2 metres; nodes are generally hairless. Slender seed stems grow to 45 cm. 1–3 racemes, usually 2, 2–10 cm long, on a many-jointed rachis. Spikelets paired, one sessile and one stalked. Spikelets are all very close together and overlap each other. Only the sessile spikelet has an awn, 1–2.5 cm long. It is native to India, Sri Lanka, Myanmar, Thailand, Indonesia and Papua New Guinea.

It may be planted from seed or stolons. Seed is awned and hence difficult to harvest and clean mechanically. De-awned seed is less likely to clump but the spiralled awn may help the seed to bury itself when wetted. Not easy to establish from seed, although in time it would spread from a relatively low density to form a close sward under suitable conditions. Planting stolons with nodes gives faster establishment but requires follow-up rainfall and is more labour intensive.

Dry matter production around 10–12 t/ha/year DM but very poor production during the dry season. In Fiji, production is highest in March at about 1,000 kg/ha/week and lowest from July–September at about 200 kg/ha/week. In Guadeloupe, it produced up to 40 kg/ha/day DM during the wet season, but none during the 5 months of dry season. Animal production up to 100 kg/ha/yr of LWGs from unimproved grassland at a stocking rate of 2.5 steers/ha may be possible; 150 kg/ha/yr with legumes (sown *Macroptilium atropurpureum* and *naturalised Desmodium heterophyllum*) and superphosphate. Animals may lose weight during the dry season because of lack of growth of grass.

P. amaura is also known as Jawa grass, Batiki Bluegrass, Indian muraina grass, the name *P. amaura* (Buse) O. Kuntze experienced a considerable taxonomic evaluation producing a numerous names more than 20 ones, and all were considered as illigimite ones, it should be named as *Polytrias indica* (Houtt.) Veldkamp. It is native to West Africa (from Senegal to Cameroon), Seychelles, the Indian Subcontinent, southern China, Southeast Asia, New Guinea, Fiji, and Micronesia. It is also cultivated as a lawn grass and in Indonesia is very popular as soccer field.

Table 5. The composition of plant species under the *V. nilotica* canopy 3 months after treatments of triclopyr to control *V. nilotica* and herbs as well as shrubs and planted with grasses (*D. caricosum* and *P. amaura*) with and without fertilizer (compost)

No	Species	CD+	CD-	CP+	CP-	BD+	BD-	BP+	BP-
		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	*Abelmuchus ficulneus*	0	0	0	0.05	0.05	0	0	0
2	*Abutilon indicum*	0.35	0.1	0	0.1	0	0.40	0.10	0.6
3	*Acacia nilotica seedling*	0.20	0.20	0.15	0.25	0.15	0.05	0.10	0.2
4	*Acalypa indica*	0	0	0	0	0.2	0	0	0
5	*Achyranthes aspera*	1.75	0.80	1.0	0.15	1.35	1.2	1.15	1.6
6	*Achyranthes sp*	0	0	0.05	0.05	0.25	0	0	0
7	*Aeschiomene americana*	0.20	0.1	0	0.30	0.05	0	0	0
8	*Azima sermentosa*	0	0	0	0	0	0	0.05	0.05
9	*Azadirachta indica*	0.1	0.05	0.15	0.05	0.6	0.35	0.45	0.3
10	*Brachia reptans*	39.65	42.65	38.05	42.40	35.85	27.60	22.75	25.45
11	*Calopogonium mucunoides*	0.05	0.30	0	0.05	0.05	0.10	0	0.1
12	*Capparis sepiaria*	0	0	0	0	0.05	0	0	0
13	*Chloris dolichostachya*	0.50	0.40	3.35	0.9	1.65	2.15	1.4	0.9
14	*Chromolaena odrata*	0	0	0.05	0	0	0	0	0
15	*Cleome aspera*	0.30	0	0.05	0	0	0.1	0.05	0.15
16	*Columella trifolia*	0.05	0	0.25	0.2	0.05	0	0	0
17	*Corchorus sp.*	0.15	0.3	0.25	0	0.4	0.1	0.3	0.15
18	*Bidens biternata*	11.30	13.4	5.85	11.55	9.39	3.55	3.25	8.70
19	*Eleuntheranthera ruderalis*	11.45	10.10	7.55	14.60	13.95	9.40	3.25	6.25
20	*Euphorbia hirta*	0.05	0	0	0	0	0	0	0

No	Species	CD+	CD-	CP+	CP-	BD+	BD-	BP+	BP-
		(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**
21	*Hedyotis corimbosa*	0.05	0.1	0.05	0.05	0.40	0.55	1.7	0.65
22	*Hisbiscus penduriformis*	0.25	0	0.05	0	0	0	0.1	0.1
23	*Hyptis suaveolens*	1.00	0.70	0.25	0.55	0.10	0.40	0.1	0.05
24	*Hyptis sp*	0	0	0	0	0	0	0	0.2
25	*Indigofera sumatrana*	0	0	0	0	0	0.05	0	0.05
26	*Jatropha gossypifolia*	0	0.50	0.05	0	0	0	0	0
27	*Merremia emarginata*	0	0	0.70	0.45	2.15	0.1	0	1.05
28	*M. gemella*	4.9	3.35	1.6	2.5	4.25	3.95	3.50	2.8
29	*Melothria moderaspatama*	0	0	0	0	0	0.05	0	0.05
30	*Mimosa invisa*	0	0.15	0.3	0.15	0.2	0.05	0.1	0
31	*Ocimum americanum*	0.25	0.05	0.05	0	0.55	0.70	0	0.4
32	*Opliemenus burmanii*	2.40	0	0	0	0	2.65	5.25	4.25
33	*Phylanthus urinaria*	0.05	0	0.05	0.1	0.05	0.05	0.15	0.15
34	*P. vareigatus*	0.15	0.1	0.15	0.15	0.35	0.25	0.30	0.05
35	*Portulaca oleraceae*	0	0	0	0	0.10	0.05	0.05	0.05
36	*Randia spinosa*	0	0	0	0	0	0.05	0	0
37	*Sida acuta*	0.05	0.05	0	0	0	0	0.05	0.05
38	*Sida cordifolia*	0	0	0	0	0	0	0	0
39	*Thephesia lampas*	1.05	1.95	1.85	1.65	0.45	0.40	0.30	0.35
40	*Vervonea cymosa*	0	0	0	0	0.20	0.25	0.25	0.25
41	*Wisadula periplocifolia*	0	0.05	0	0	0	0	0	0
	Total	**74.35**	**75.40**	**48.05**	**76.25**	**72.29**	**54.50**	**44.70**	**54.3**

NB. **C** = cut with a chainsaw, and the stump was immediately brushed with triclopyr at 1.5 ml/l00 ml dissolved in diesel oil. **B** = brushed with triclopyr at 1.5 ml/l00 ml dissolved in diesel oil at the base of a standing tree. **D** = planted with *Dicanthium caricosum*. **P** = planted with *Polytrias amaura*. + = fertilized with a bucket of compost (100 gr)/planted sod. - = without fertilizer. The plot of each unit treatments = 7 x 7 m^2.

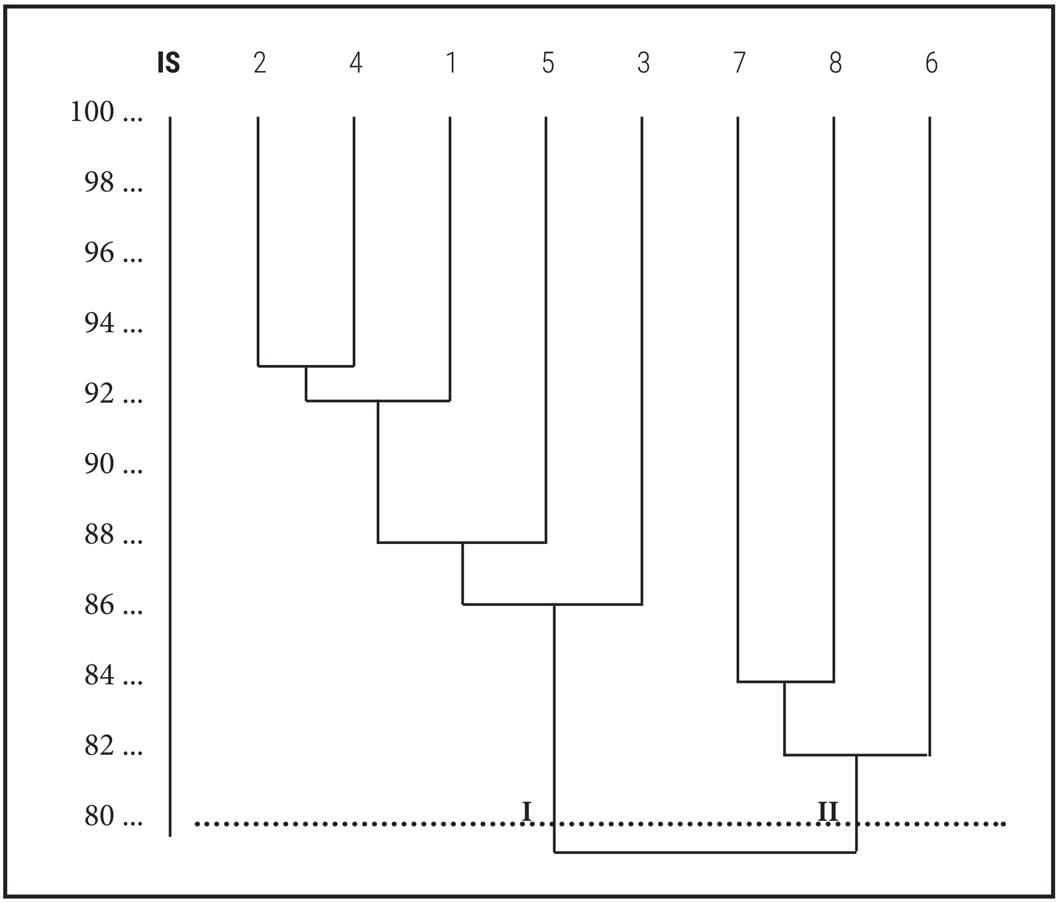

Figure 2. Dendrogram of 8 vegetation compositions under *V. nilotica* canopy 3 months after foliar application of GARLON 670 EC at 670 g.ai/ha.

Table 6. The grass coverage as affected by the application of triclopyr at 1.5 ml/l00 ml on *V. nilotica* tree

No	Treatments	% grass coverage
1	*V. nilotica* cut and brushed on stump	43.12
2	*V. nilotica* brushed standing	27.58
3	LSD (5%)	13.27

Table 7. Means of biomass of *C. caricosum* and *P. amaura* (g/m^2) as affected by the application of triclopyr at 1.5 ml/l00 ml dissolved in diesel oil to control *V. nilotica*

Application of 1.5 ml/l00 ml dissolved in diesel oil	*D. caricosum*		*P. amaura*	
	With compost	Without compost	With compost	Without compost
Brushed on stump	971.25	703.25	574.25	547.75
Brushed at the tree base	831.75	835.75	713.75	646.75

Further Sugesstion of Management

Recent analysis of vegetation in the experimental plots 3 years after treatments displayed an improvement in the vegetation composition toward the establishknet of palatable grassess mainly *D. caricosum* and *P. amaura* (See Table 8). The growth of *D. caricosum* in both condition wherther *V. nilotica* was controlled by brushing 1% garlon on cut stump or standing stem the population was similar, while that of *P. amaura* was slightly better under open condition facilitated by cuttings *V. nilotica* stump. From this analysis the vegetation was dominated by grasses, especially *B. reptans*, and reintroduced *D. caricosum*, and *P. amaura*. However *V. nilotica* grew again from seed bank in the soil.

There are three problems after controlling *V. nilotica* that must be addressed, i.e. (1) The growth of broadleved weeds, these included climbers such such *Desmodium*, but also those having undergrown stolon such as *Flemengia lineata*, beside common fast growing weeds such as *B. biternata, V. cymosa, T. lampas*. These shrubs are able to grow in a dense population restricting the movement of animals and humans. These weeds must be sufficiently controlled. (2) The second problem is the coming back of *V. nilotica* through the germination of seed bank. It is better to kill *V. nilotica* when still young, before reaching its generative stage. The last one is (3) the growth of grasses. The introduced grasses must be prevented from being grazed by herbivores before strong enough. In our discussion CABI suggested not only to utilize the stump of *V. nilotica*, but also branches to prevent the herbivores from grassing the planted grasses until they are strong enough to stand the grazing.

During the project it was also noticed that some bruchids also attacked the seed of *V. nilotica*, it may be researched and capitalized its function to reduce *Acacia* seed from germinating. Risk analysis on invasive plant species in Baluran National Park have been done, and it should be utilized in the management of the park as directed by Aichi Biodiversity Target no 9. It is necessary then to bring to the attention of the related Directorate General to work in line with the time table as directed by Aichi Biodiversity Target No.9.

The Directorate General must be able to provide the necessary fund to carry out activities as directed by Aichi Biodiversity Targtet No 9. While the government may have difficulties in giving priority to the management of Invasive Species, the Central Research of Forestry and Innovation should display its "innovativeness" by, for example utilizing the wood of *V. nilotica* as a materials that may be utilized for making charcole . It was practised in the past and technically very good, the only necessary step, now, is to regulate and legalise the practise the monetary benefit of which must be utilized to control *V. nilotica* further. The shortage of funds experienced by the government should not become reasons for not managing these invasive plant species, because they will grow and expands further to inflick a greater damage to the society.

Table 8. Vegetation of Bekol savanna after *V. nilotica* control through mechanical cutting of trees followed by brushing garlon at 1% disolved in diesel oil, and broadleaved weeds were also controlled with foliar application of garlon at 1 lt/ha using applied 400 lt water wing o.2 a knapsack sprayer calibrated to deliver a spray volume of 400 lt/ha

N0	Species	***V. nilotica* was brushed with Garlon 1% dissolved in diesel oil**		
		Brushed on cut stump	**Brushed on standing stump**	**Means no/m²**
1	*Abutilon hirtum*	0.2	0.6	0.4
2	*Abelmoschus ficulneus*	0..01	0.06	*
3	*Acacia leucophloea*	0.01		*
4	*Acacia nilotica*	2.2	1.0	1.6
5	*Acalypha indica*	0.03	0.6	0.3
6	*Achyranthes aspera*	0.4	0.8	0.6
7	*Aeschinomene americana*	0.5	0.2	0.3
8	*Ageratum conyzoides*	0.05	0.02	*
9	*Azadirachta indica*	0.41	2.5	1.5
10	*Azima sarmentosa*	0.02	0.2	0.1
11	*Bidens biternatta*	3.46	7.2	5.3
12	*Boerhavia erecta*	0.01	0.05	*
13	*Brachiaria reptans*	89.4	33.7	61.55
14	*Calliandra* sp.	0.4	1.6	1.0
15	*Calotropis gigantea*			
16	*Capparis sepiaria*	0.04		*
17	*Cayratia trifolia*	0.1	0.1	0.1
18	*Clitoria ternatea*		0.04	*

N0	Species	*V. nilotica* was brushed with Garlon 1% dissolved in diesel oil		
		Brushed on cut stump	Brushed on standing stump	Means no/m²
19	*Commelina benghalensis*		0.01	*
20	*Corchorus aestuans*	0.3		0.15
21	*Corchorus olitorius*	0.9	0.3	0.5
22	*Corypha utan*	0.3	0.07	0.1
23	*Casia obtusifolia*	0.01		*
24	*Desmodium dichotomum*	0.9	0.7	0.8
25	*Dichantium caricosum*	9.4	9.4	9.4
26	*Dichrostachys cinerea*	0.02		*
27	*Dicliptera canescens*	0.06	0.7	0.3
28	*Echinochloa colonum*			
29	*Eleutheranthera ruderalis*	3.8	4.4	4.1
30	*Eragrostis unioloides*	0.05		*
31	*Euphorbia heterophylla*	0.05		*
32	*Euphorbia hirta*	0.01	0.1	*
33	*Flemingea linneata*	0.02		*
34	*Hedyotis pterita*	0.9		0.45
35	*Hibiscus panduriformis*	1.7	2.4	1.6
36	*Hibiscus sp.*	2.2	0.8	1.5
37	*Hibiscus vitifolius*	0.03	0.01	*
38	*Hyptis suaveolens*	0.9	1.75	1.3
39	*Hyptis* sp.	0.01	0.01	*
40	*Indigofera pratensis*	0.05	0.7	0.3
41	*Jacquemontia paniculata*	3.25	3.9	3.1
42	*Jatropha gossypifolia*	0.8	0.01	0.4
43	*Lantana camara*		0.01	*
44	*Merremia emarginata*	1.0	0.4	0.7
45	*Mimosa invisa*	0.2	0.2	0.2
46	*Mukia maderaspatana*		0.16	0.8
47	*Ocimum americanum*	2.0	2.0	2.0
48	*Passiflora foetida*		0.02	*
49	*Polytrias amaura*	16.8	10.2	13.5
50	*Phyllanthus maderaspatensis*	0.5	0.7	0.6
51	*Phyllanthus urinaria*	0.03	0.03	*
52	*Phyllanthus virgatus*	4.7	3.6	4.1
53	*Randia spinosa*	0.01		*
54	*Sida cordifolia*	0.2	0.4	0.3
55	*Synedrella nudiflora*			
56	*Vernonia cymosa*	8.3	.2.5	5.4
57	*Wisadula periplocifolia*		0.01	*
58	*Ziziphus mauritiana*	0.04	0.02	*
59	*Ziziphus oeonopholia*	0.02	0.01	*
60	*Number of species*	50	46	

CONCLUSION

It was concluded that the method of controlling *V. nilotica* by chopping *V. nilotica* trees during the dry season leaving only 10 cm stump left on the ground and directly. brushing 1 % Garlon 670 EC dissolved in diesel oil on left stump was good to provide the growth of planted grasses. Especially that of *D. caricosum*. It was also suggested to utilize the leaves of *V. nilotica* as herbage for herbivores while utilizing the wood for charcoal production.

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HABITAT SUITABILITY AND NICHE INTERACTION BETWEEN THE INVASIVE SNAIL *ACHATINA FULICA* AND ITS BIOCONTROL FLATWORM *PLATYDEMUS MANOKWARI* IN SOUTHEAST ASIA

Britney Ann P. Yu[1]*, Geofe O. Cadiz[1], Mary Joyce Flores[1], Brisneve Edullantes[1]

[1]Department of Biology and Environmental Science, College of Science, University of the Philippines Cebu, Cebu City, Cebu 6000, Philippines

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***Corresponding author:**
bpyu@up.edu.ph

ABSTRACT

Bioinvasions are increasingly disrupting community structures worldwide, especially as the climate remains unstable. In invaded areas, biocontrol agents are often introduced to help manage the spread of invasive species. However, these agents can proliferate and threaten non-target organisms without thorough evaluation. We assessed the niche dynamics between invasive *Achatina fulica* (Giant African Snail) and its biocontrol agent, the *Platydemus manokwari* (New Guinea Flatworm), in Southeast Asia. Species occurrence and environmental data were used to model the habitat suitability of both species in the present and future climate scenarios using ecological niche modeling with the MaxEnt algorithm. These models predicted 25.9% and 42.0% of the current conditions as suitable and 73.8% and 57.8% as unsuitable for *A. fulica* and P. manokwari, respectively. There was a predicted steady increase in suitable areas and a gradual decrease in *A. fulica's* unsuitable areas as the carbon emissions are predicted to increase. Moderate to high niche overlap of 61.2% to 83.4% was expected between the species under different climate scenarios. Predicting the suitable areas for invasive species and their niche interaction with other species, especially in the context of climate change, will aid in identifying vulnerable areas for conservation and potential outbreaks of infectious diseases.

Keywords:
climate change, niche expansion, niche overlap, ecological niche modeling, MaxEnt.

INTRODUCTION

Biological invasions are threatening the biodiversity of ecological communities all over the world. Invasive species continue to endanger the survival of numerous populations as their effects cascade through the ecosystem. Hence, it is essential to control these biological invasions to prevent biodiversity loss. Strategies have been implemented to regulate the invasive species' populations through chemical, mechanical, and biological methods. Mechanical control involves the physical collection of the target species for eventual disposal. While this method is effective for small-scale applications, it requires a large workforce, making it expensive. Meanwhile, chemical control uses chemicals to remove target species, harming the surrounding organisms and the environment (Weidlich et al., 2020).

Among the strategies, the biological method is the most sustainable way (Clewley et al., 2012). Classical biological control (hereafter referred to as biocontrol) involves natural enemies to regulate the population of invasive species. As the selected biocontrol agent exists in the same environment as its target species, its niche may expand or contract, demonstrating the competitive exclusion principle. Using biocontrol agents that feed exclusively on their target species will reduce the probability of disturbing other organisms in the vicinity. In contrast, predators introduced to infested areas without prior evaluation of their host specificity typically eradicate native species (Gerlach et al., 2021). Such is the case with the Euglandina rosea (Rosy wolf snail) and *Platydemus manokwari* (New Guinea flatworm) in controlling the global population of the giant African snail (*Achatina fulica*). The presence of these animals led to the drastic reduction of the native snail population in Japan and Hawaii, making it imperative to assess potential biocontrol agents before their release to invaded areas.

Laboratory and field experiments are typically used to evaluate the interaction between species. However, laboratory conditions may not simulate the environment in the natural setting. Field experiments provide this advantage but can unintentionally release potentially invasive species instead. A more recent approach is the use of ecological niche modeling (ENM) to determine the niche interaction between two species by considering their distribution and the set of environmental conditions they need for optimal survival and reproduction (Valencia-Rodríguez et al., 2021). The advent of ENM has made it more convenient to investigate invasive species' niche dynamics and their respective target hosts. However, studies investigating the niche interaction between biocontrol agents and their target invasive species are still limited.

The niche concept has long been a struggle for researchers to define due to its multifaceted nature, as interpreted by previous authors, such as Grinel, Elton, and Hutchinson (Sales et al., 2021). In their work, Sales et al. (2021) dissected this concept in terms of key components, including but not limited to the species' relationship with the environment, the presence of competition, and the scope of niche in research. Habitat suitability modeling studies utilize species distribution under specific environmental conditions to predict habitable areas at a given time. Recently, these studies involve a wide range of relevant ecological issues, such as the conservation of endemic and endangered species (Paul & Samant, 2024), the invasion risk of invasive species (Ahn et al., 2023), and the effect of climate change on their distribution (Mothes et al., 2020). When the needs of two or more species overlap, their niche will potentially influence the other, resulting in habitat shifts (Chen et al., 2024).

Achatina fulica, or the Giant African Snail, is a terrestrial mollusk native to East Africa. They are hermaphroditic gastropods that produce approximately 1,200 eggs annually. It was eventually introduced to the Southeast Asian region through intentional and accidental introductions. Presently, *A. fulica* continues to feed on a wide variety of vegetation. This characteristic is detrimental to residential areas with ornamental plants, particularly agricultural farms (Ramdwar et al., 2018). *Platydemus manokwari*, on the other hand, are raptorial flatworms introduced to *A. fulica's* invaded areas as biocontrol agents. Initial research considered these flatworms effective, but there was no long-term evaluation of their environmental impact (Muniappan et al., 1986). The overwhelming evidence of *P. manokwari*'s negative impacts on the native fauna is highly substantial in the literature (Muniappan et al., 1986; Gerlach et al., 2021). In their review, Gerlach et al. (2021) strongly asserted terminating any further introductions of *P. manokwari* to control *A. fulica*.

Aside from threatening biodiversity, ***A. fulica*** and *P. manokwari* are also hosts to parasitic nematodes that carry potential diseases to humans. Studies from the Philippines, Thailand, Hawaii, and the United States found the rat lungworms Angiostrongylus cantonensis and A. malaysiensis present in these two species (Hollingsworth et al., 2013; Chaisiri et al., 2019). Eosinophilic meningitis, the primary disease associated with the Angiostrongylus genus, could be lethal to humans as it can cause neurological defects and even death. With the ongoing climate crisis, determining possible invasion areas by these species could be unpredictable. This study aims to assess the niche interaction between the invasive snail *A. fulica* and its biocontrol agent *P. manokwari* in the Southeast Asian region in the current and future climate scenarios. Specifically, this study aims to (a) assess the current habitat suitability of both species in Southeast Asia, (b) compare the current and future habitat suitability for both species and (c) evaluate the niche interaction between the two species in all climate scenarios using niche overlap and niche dynamics analysis.

MATERIALS AND METHODS

A correlative ecological niche modeling approach was used to predict the habitat suitability and niche of *A. fulica* and *P. manokwari* in the Southeast Asian region under current and future environmental conditions.

Study Species

In this study, the species of interest are the invasive Giant African snail (*A. fulica*) and the invasive biocontrol agent, which is the New Guinea flatworm (*P. manokwari*). *A. fulica* is a terrestrial mollusk of the Achatinidae family with varying patterns of yellow to brownish striations around its shell. Due to their broad environmental tolerance, *A. fulica* snails are now widespread pests in agricultural lands; they consume crops such as grains, rice crops, cabbages, and even other snails. To control the *A. fulica* invasion, *P. manokwari* was introduced in *A. fulica*'s invaded areas. *P. manokwari* is a flatworm belonging to the family Geoplanidae characterized by its dark brown to black coloration, a light brown band running along its body, and a pale underbelly.

Study Site

This study analyzed the occurrence of the two species in Southeast Asia (11°S–28°N and 92–141°E) as shown in Figure 1. Southeast Asia comprises eleven countries in the mainland and island zones, including Thailand, Malaysia, Cambodia, Myanmar, Brunei, Timor-Leste, Indonesia, Singapore, Laos, Vietnam, and the Philippines. The climate is tropical, with average temperatures above 25°C all year round; Asian monsoons bring significant rainfall to these tropical areas. Southeast Asia, known for its biodiversity hotspots, faces severe threats to its ecosystems despite being home to several of them.

Occurrence and Environmental Data

The occurrence data for *A. fulica* and *P. manokwari* were downloaded from online biodiversity databases, particularly the GBIF (Global Biodiversity Information Facility at https://www.gbif.org) and iNaturalist (https://www.inaturalist.org/). Criteria on the type of data, basis of observation, and geographic location were filtered out before downloading. Only presence data from Southeast Asian countries gathered through human observation, preserved specimens, and species were downloaded from GBIF. Likewise, only verifiable and research-grade data were downloaded from iNaturalist. Data from published studies involving the occurrence of the two species in Southeast Asian countries were also included, such as from the works of Chaisiri et al. (2019), Huang et al. (2019), and Muniappan et al. (1986). The occurrence records were georeferenced using QGIS 3.24.2 (QGIS Development Team, 2009). The raw data now includes 3984 *A. fulica* and 199 *P. manokwari* occurrence points.

The species occurrence data were processed before modeling using R 4.2.0 (R Core Team, 2020). Spatial filtering removed data points found outside the study area to ensure that the remaining points were within the geographic extent (Minimum Longitude: 92.208; Minimum Latitude: 12.192; Maximum Longitude 141.000; Maximum Latitude: 28.542). The duplicate coordinates were then filtered out using the R package CoordinateCleaner. Spatial thinning, using the spThin package, was performed to reduce sampling bias while retaining the highest number of substantial data points. After cleaning the data, 1386 and 124 occurrence points remained for *A. fulica* and *P. manokwari*, respectively (Figure 1).

The environmental variables in the study consist of 19 bioclimatic and 10 soil variables, which were also used in other related studies (Banerjee et al., 2020; Feng et al., 2021). The bioclimatic variables were acquired from WorldClim ver 2.1 with a spatial resolution of 30-arc seconds (~ 1 km2) taken from 1970 to 2000 (Fick & Hijmans, 2017). These are derived from monthly measurements of temperature and rainfall alongside their derivations in terms of annual trends, seasonality, and extreme conditions. Meanwhile, the soil data sets were downloaded from ISRIC (International Soil Reference and Information Center) SoilGrids (https://www.isric.org/explore/soilgrids). The soil variables are described in Table 1. The soil parameters are important since they influence the biology of the study species, as both are land dwellers.

The environmental variables were then cropped to the Southeast Asian extent using the raster package in R. The resolution and extent of the species occurrence and environmental variables should be the same to avoid any discrepancy. The autocorrelation between the environmental variables was also prevented using Pearson's correlation coefficient. Highly correlated variables below the threshold were assessed; only one among two highly correlated variables (> 0.75) was retained for modeling based on their ecological significance to the species. After analyses, 10 environmental variables were retained for niche modeling (Table 1).

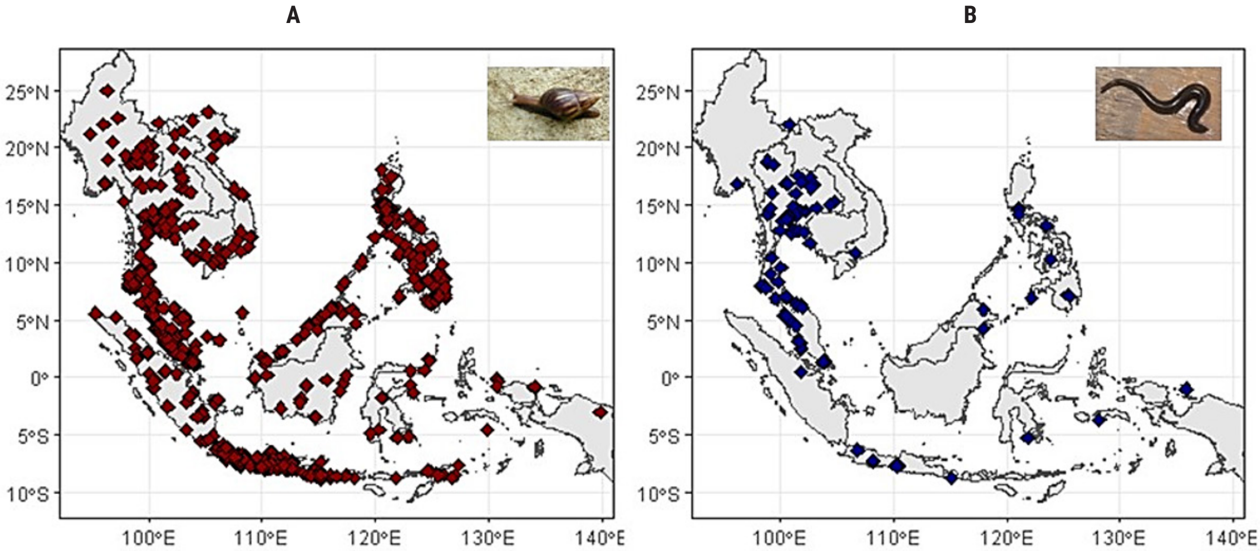


Figure 1. Occurrence data of (A) *Achatina fulica* and (B) *Platydemus manokwari* in Southeast Asia. Photo source of the two species:
https://indiabiodiversity.org/species/show/237254;
https://commons.wikimedia.org/wiki/File:Peerj-297-fig-1_Platydemus_manokwari.png#filelinks)

Table 1. List of environmental variables used in the model development for *Achatina fulica* and *Platydemus manokwari*.

Variable code	Variable	Units	Source
bio1*	Annual mean temperature	°C	Wordclim ver 2.1 (Fick and Hijmans, 2017)
bio2*	Mean diurnal range	°C	
bio3	Isothermality	°C	
bio4	Temperature seasonality	°C	
bio5	Maximum temperature of warmest month	°C	
bio6	Maximum temperature of coldest month	°C	
bio7	Temperature annual range	°C	
bio8	Mean temperature of wettest quarter	°C	
bio9	Mean temperature of driest quarter	°C	
bio10	Mean temperature of warmest quarter	°C	
bio11	Mean temperature of coldest quarter	°C	
bio12*	Annual precipitation	mm	
bio13	Precipitation of wettest month		
bio14	Precipitation of driest month	mm	
bio15	Precipitation seasonality	mm	
bio16	Precipitation of wettest quarter	mm	
bio17	Precipitation of driest quarter	mm	
bio18*	Precipitation of warmest quarter	mm	
bio19*	Precipitation of coldest quarter	mm	
bdod*	Bulk density of the fine earth fraction	kg / dm^3	ISRIC SoilsGrid (Poggio et al., 2021)
cfvo*	Volumetric fraction of coarse segments	cm^3 / dm^3	
nitrogen	Total nitrogen (N)	g / kg	
ocd*	Organic carbon density	kg / m^3	
phh2o*	pH of water in soil		
sand	Sand (>0.05 mm) in fine earth	%	
silt	Silt (0.002 - 0.05 mm) in fine earth	kg / m^3	
ocs	Organic carbon stocks	kg / m^3	
soc*	Soil organic carbon in fine Earth	g*kg^-1	

*Variables used in the final models to produce habitat suitability and niche interaction results

Modeling the Current Distribution of *A. fulica* and *P. manokwari*

The Maximum Entropy (MaxEnt) algorithm was used to model the habitat suitability of *A. fulica* and *P. manokwari*. MaxEnt is widely used to model species distribution, considering several predictor variables and a wide range of species occurrence data points. However, it is susceptible to sampling bias. The background points were estimated using the RasterStack object of the selected environmental variables and species occurrence data to account for sampling bias. Separate bias files were created for *A. fulica* and *P. manokwari* using the MASS package's two-dimensional kernel density estimation. The bias files in .asc format, alongside the environmental variables and species occurrence data, were inputted into the MaxEnt software version 3.4.4 (http://biodiversityinformatics.amnh.org/open_source/maxent/). To improve predictive accuracy, the parameters were set according to the results from the ENMevaluate function of the ENMeval package. This function considers the species occurrence and the stack of the 10 selected environmental layers using the "randomkfold" cross-validation method. The parameter settings with the lowest delta AICc (delta AICc = 0) were selected, particularly involving the linear, quadratic, hinge, product, and threshold features with a regularization parameter of 0.5. The maximum number of background points was set to 10,000 as more than 50,000 potential background points for Southeast Asia. The occurrence data were randomly divided into two groups, with 75% allocated for training the data and 25% for testing the models using the bootstrapping method with ten replicates per model. Each environmental variable's percentage contribution and relative importance were assessed using the jackknife test available in the MaxEnt software. The rest of the parameters were set to default. Model accuracy is determined through the area under the receiver operating characteristic curve score (AUC ROC), with a value closer to 1 being the most accurate (Shabani et al., 2018).

Predicting the Current and Future Habitat Suitability

The species distribution models using the current environmental conditions were projected to future climate scenarios for 2070. The future bioclimatic variables with a 30-second spatial resolution were downloaded from CMIP5 (Coupled Model Intercomparison Project Phase 5) using the Beijing Climate System Model (BCC-CSM) as the general circulation model (GCM). The soil variables in the current condition were retained due to the unavailability of future simulations, assuming the soil conditions do not change in the future. The future climate scenarios were based on four Representative Concentration Pathways (RCPs) set by the Intergovernmental Panel on Climate Change (IPCC), which are the RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5 (van Vuuren et al., 2011). RCPs are projections that take into account current data on energy, land use, and greenhouse gas emissions; the higher the RCP level, the higher the simulated emissions values. According to Table 2, the study site was classified as either unsuitable or suitable based on the maximum test sensitivity plus specificity logistic threshold of the models. The present and future habitat suitability for each species were mapped using the QGIS 3.20 software (QGIS Development Team, 2009).

Niche Overlap and Statistical Analysis

The niche overlap between *A. fulica* and *P. manokwari* in the current and future climate scenarios was determined using the PCA-env technique adapted from Broennimann et al. (2012). This technique considers the density of species occurrences while accounting for the 10 selected environmental variables and the available background points. A hundred replicates of niche overlap over the density distributions of both species were measured and then visualized using histograms. Niche overlap indices used in this study were the Schoener D and Hellinger I indices, wherein values closer to 0 signify no overlap, while 1 represents identical niche models. Niche similarity and niche equivalency were used to determine if there was a statistical difference between the niches of the two species. Niche similarity analyzes whether the two interest niches have higher similarity than is expected by chance. In contrast, niche equivalency determines how constant the niche overlap is over randomized species occurrence points. All niche overlap analyses were then analyzed using the ecospat package in R ver. 4.1.1 (Warren et al., 2021).

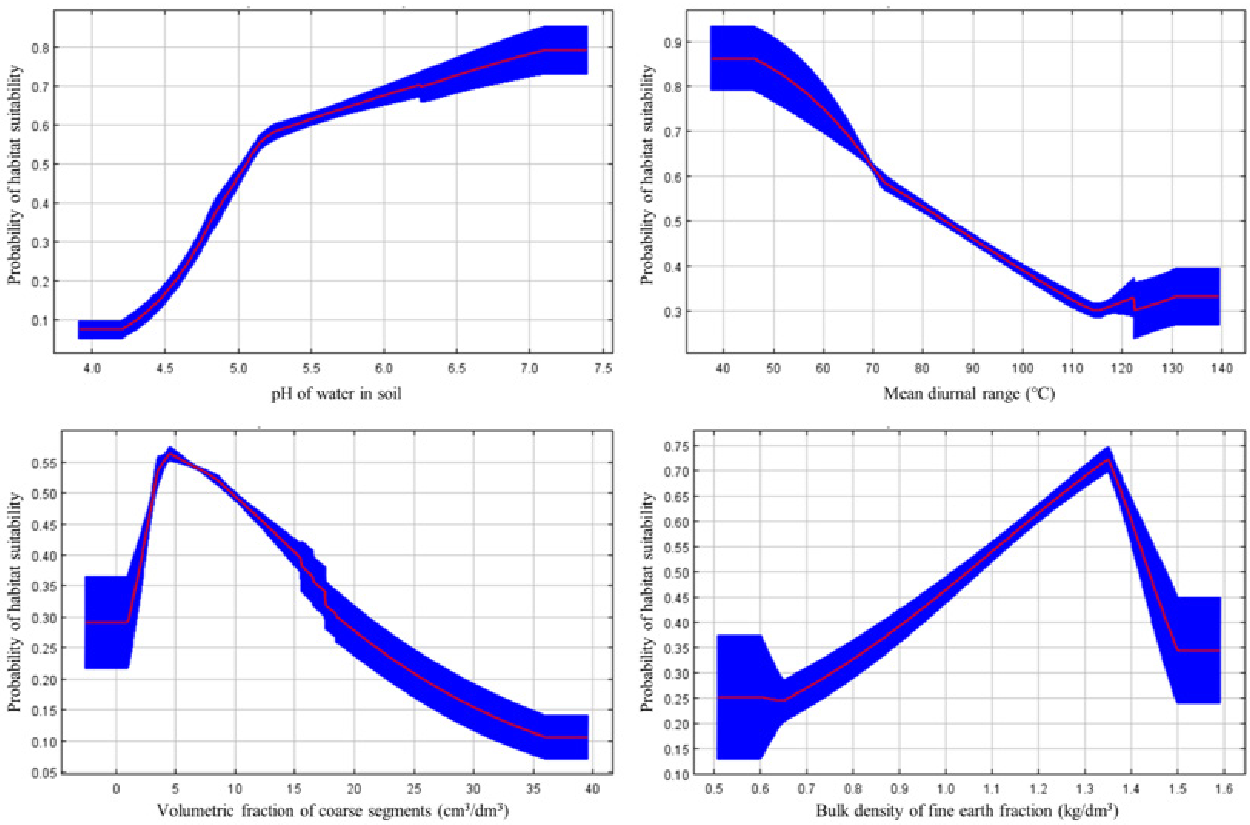
RESULTS

Model Evaluation and Environmental Variables

The AUC ROC score for *A. fulica* and *P. manokwari* was 0.78 ± 0.01 and 0.76 ± 0.05, respectively, indicating a moderate model accuracy (Shabani et al., 2018). After modeling, the response curves of environmental variables with a percent contribution of more than five percent were produced. Table 2 shows the highest contributing variables for *A. fulica*, which includes phh2o (34%), bio2 (15.4%), cfvo (12.8%), bdod (11.7%), and soc (10.2%). Additionally, it shows that bio2 was the highest contributing variable to the *P. manokwari* model with a 39.9% contribution, followed by phh2o (31.9%), bio1 (9.2%), and bio18 (6.8%). The probability of habitat suitability for both species in different variables is shown in Figures 2 and 3.

Table 2. Environmental variables for the present habitat suitability model of *A. fulica* and *P. manokwari*

Variable	*A. fulica*		*P. manokwari*	
	Percent contribution	**Permutation importance**	**Percent contribution**	**Permutation importance**
pH of water in soil	34	27.4	31.9	31.9
Mean diurnal range	15.4	5	39.9	11.3
Volumetric fraction of coarse segments	12.8	19	1	2.3
Bulk density of the fine earth fraction	11.7	16.6	3.1	5.3
Soil organic carbon in fine earth	10.2	12.3	1.7	2.8
Annual mean temperature	4.2	2.1	9.2	21.3
Precipitation of coldest quarter	4.2	5.3	2	8.8
Organic carbon density	4.2	5.9	1	0.6
Precipitation of warmest quarter	2.7	5.4	6.8	1.2
Annual precipitation	0.6	1.1	3.5	14.5



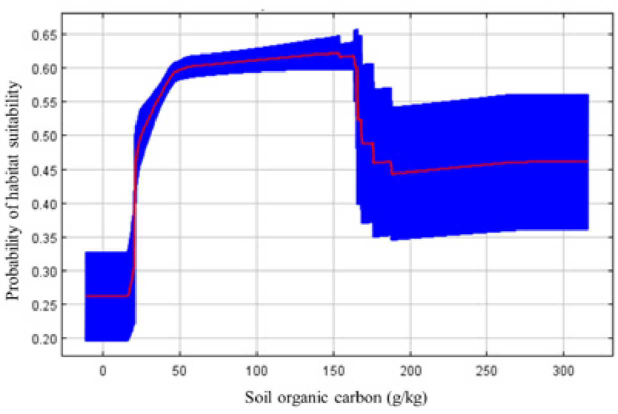


Figure 2. Response curves for each highly contributing variable to *A. fulica's* habitat suitability model. Tolerance range values are the following: 3.91 - 7.39 (phh2o), 4.0 - 100 °C (bio2), 3.25-30.8 cm^3/dm^3 (cfvo), 0.51 - 1.59 kg/dm^3 (bdod), and 15.9 - 154 g/kg (soc). Red curves are the mean probability of habitat suitability, while shaded blue regions represent the standard deviation of ± 1 for 10 replicates.

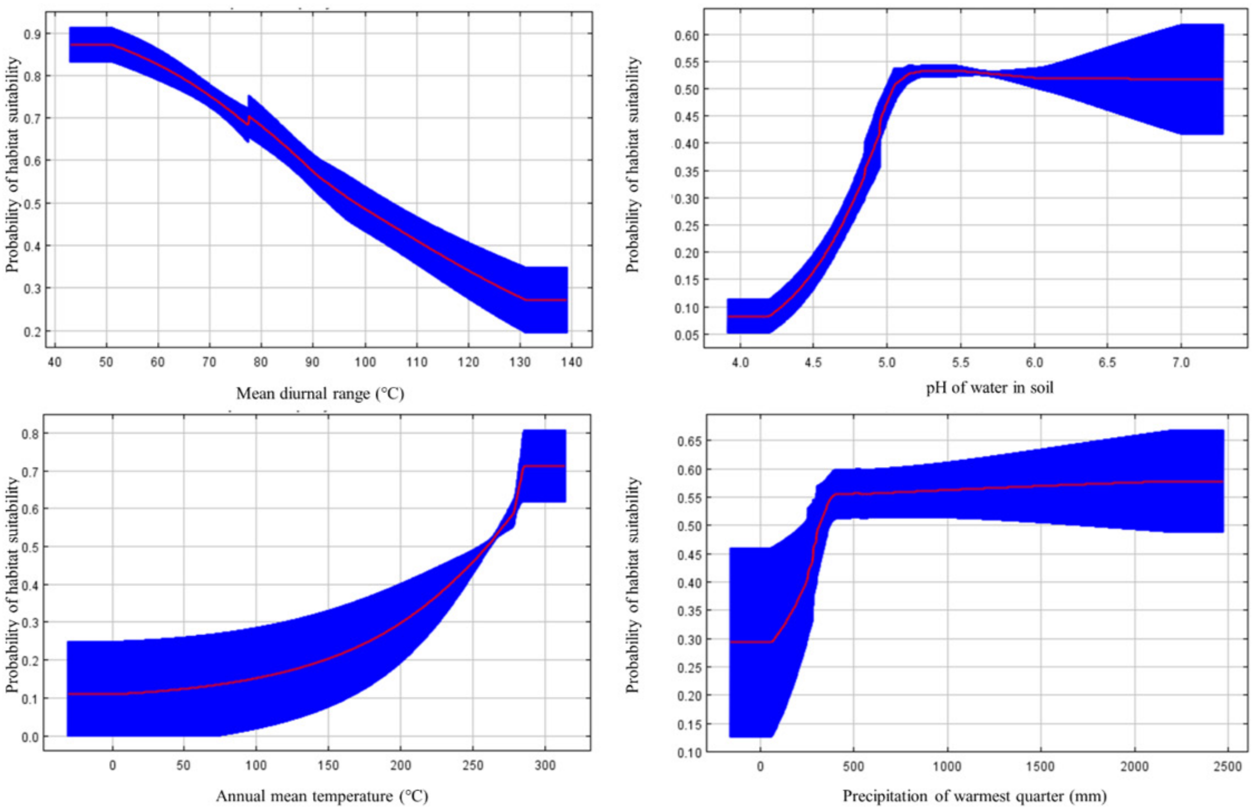


Figure 3. Response curves for each highly contributing variable to the habitat suitability model of *P. manokwari*. Tolerance range values are the following: 4.5 - 10 °C (bio2), 3.9 - 7.-0 (phh2o), 3 - 31 °C (bio1), 500 - 2500 mm (bio18). Red curves are the mean probability of habitat suitability, while shaded blue regions represent the standard deviation of ± 1 for 10 replicates.

Present Habitat Suitability

The models generated using the environmental variables in Table 2 predicted the presently suitable habitats for *A. fulica* and *P. manokwari* (Figure 4). The predicted geographical extent of suitable habitats in Southeast Asia in km2 is shown in Figure 5. Areas unsuitable for *A. fulica* were predicted to cover the majority of the region at approximately 73.8%, while only 25.9% are suitable. Predicted suitable areas include the Vietnam coasts, Myanmar, Lao, and Brunei, and a few portions of Indonesia and the Philippines. Similarly, 57.8% of the region was predicted to be unsuitable for *P. manokwari*, and only 42.0% was found suitable. Some places are commonly suitable for both species but *P. manokwari* occupies a larger geographical extent (Figure 4).

Future Habitat Suitability

The niche models generated from the present environmental conditions were used to predict the future habitat suitability for *A. fulica* and *P. manokwari*. The steady increase of suitable areas with increasing RCPs yields a gradual decrease in unsuitable areas for *A. fulica* snails (Figure 6). Particularly, there is an increase in suitable habitats of 8.5%, 17.5%, 17.5%, and 21.3% for RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5, respectively. On the other hand, the extent of suitable and unsuitable areas for *P. manokwari* was predicted to be relatively stable at RCPs 4.5, 6.0, and 8.5 compared to that of *A. fulica*. Only in RCP 2.6 was the proportion of suitable to the unsuitable area predicted at 1:3 or approximately 24.9% and 74.9%, respectively (Figure 7). Figure 8 shows the projected habitat suitability maps of both *A. fulica* and *P. manokwari* in the present and future climate conditions.

Niche Overlap and Dynamics

Moderate to high niche overlap was observed between *A. fulica* and *P. manokwari*, ranging from 61.2% to 83.4% (Table 3). Schoener's D value had little to no change in the present conditions relative to the future climate scenarios. In terms of equivalence, a significant difference between the invasive *A. fulica* and biocontrol agent *P. manokwari* was only observable in RCP 2.6. Furthermore, the niches of both species were predicted to be more similar than expected only in future climate scenarios. Under predicted current conditions, the niches were neither equivalent nor similar.

Niche dynamics were evaluated by determining the niche stability, expansion, and unfilling between the invasive species and the biocontrol agent to assess their niche interaction (Table 4). Niche stability pertains to the areas occupied by both *A. fulica* and *P. manokwari*. On the other hand, niche expansion and niche unfilling refer to areas inhabited exclusively by *A.*

fulica or *P. manokwari*, respectively. These measures of niche dynamics show one species' influence on the other's niche. The niche interaction between the two species was predicted to be relatively constant in all future climate scenarios, with no significant expansion of *A. fulica*. However, the niche unfilling by *P. manokwari* was considered significant, albeit small, in the present (p = 0.009) and future climate conditions (p = 0.009, p = 0.020, p = 0.010, = 0.010 for RCP 2.6, RCP 4.5, RCP 6.0, and RCP 8.5 respectively).

Table 3. Niche overlap indices between *A. fulica* and *P. manokwari* in present and future climate conditions.

Indices	**Climate Scenarios**				
	Present	**RCP 2.6**	**RCP 4.5**	**RCP 6.0**	**RCP 8.5**
Overlap (D)	61.2 %	61.6%	61.8%	63.1%	63.0%
Equivalence (p-value)	0.07	0.02*	0.25	0.06	0.12
Similarity (p-value)	0.05	0.01*	0.02*	0.01*	0.02*
Overlap (I)	83.1%	83.2%	83.4%	83.1%	83.4%
p-value	0.08	0.02*	0.15	0.09	0.08

** statistically significant (p-value < 0.05)*

Table 4. Niche overlap dynamics between *A. fulica* and *P. manokwari* in present and future climate conditions.

Indices	**Climate Scenarios**				
	Present	**RCP 2.6**	**RCP 4.5**	**RCP 6.0**	**RCP 8.5**
Expansion	0.68%	1.20%	1.30%	0.50%	0.74%
Stability	99.3%	98.8%	98.7%	99.5%	99.3%
Unfilling	5.00%*	4.85%*	3.18%*	5.61%*	4.68%*

**** statistically significant (p-value < 0.05)***

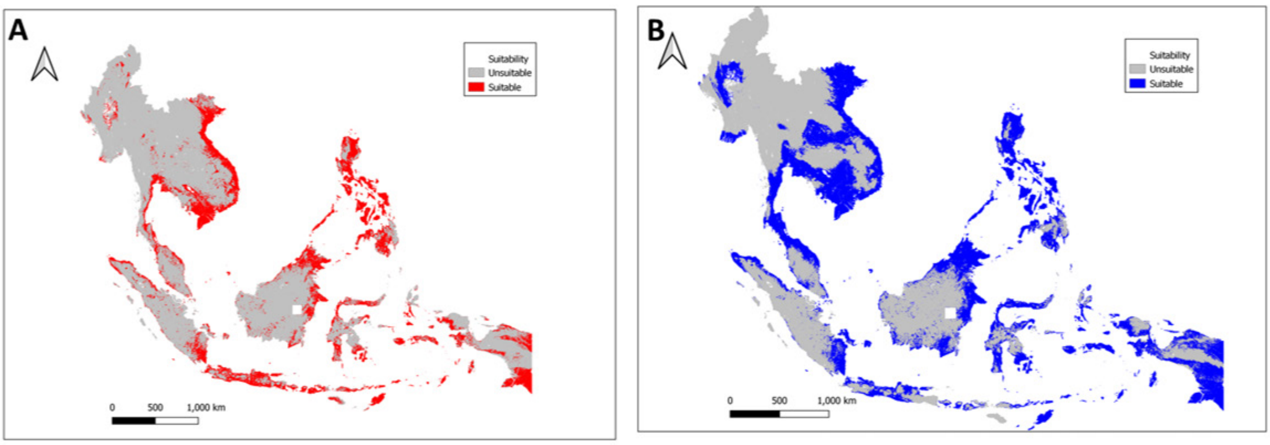


Figure 4. Present habitat suitability for *A. fulica* (A) and *P. manokwari* (B).

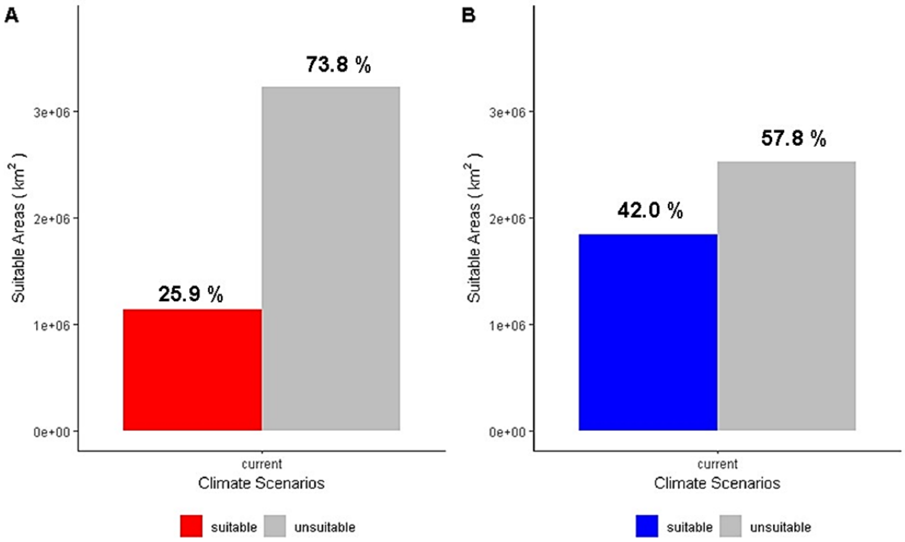


Figure 5. Areas of present habitat suitability for *A. fulica* (A) and *P. manokwari* (B) in km² relative to the whole of Southeast Asia.

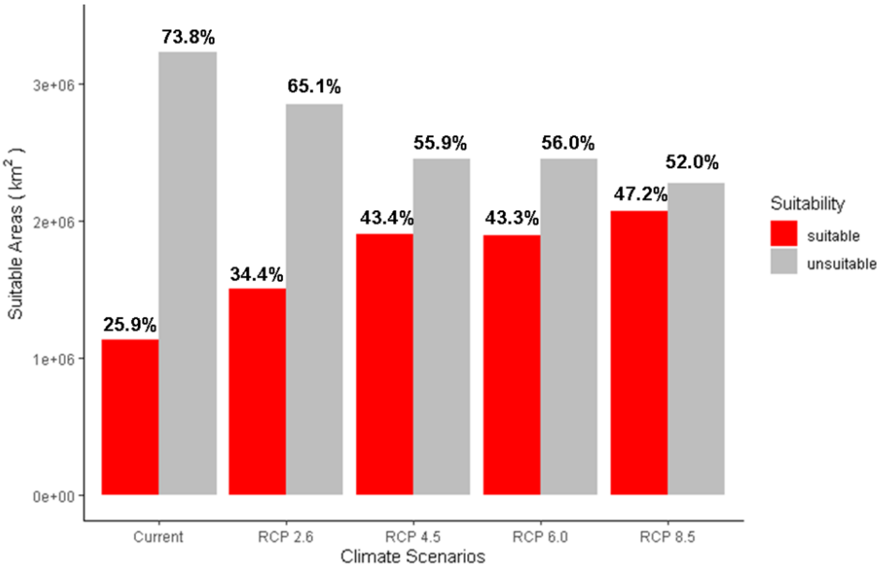


Figure 6. Areas of habitat suitability (km²) of *A. fulica* in present and future climate scenarios (2070).

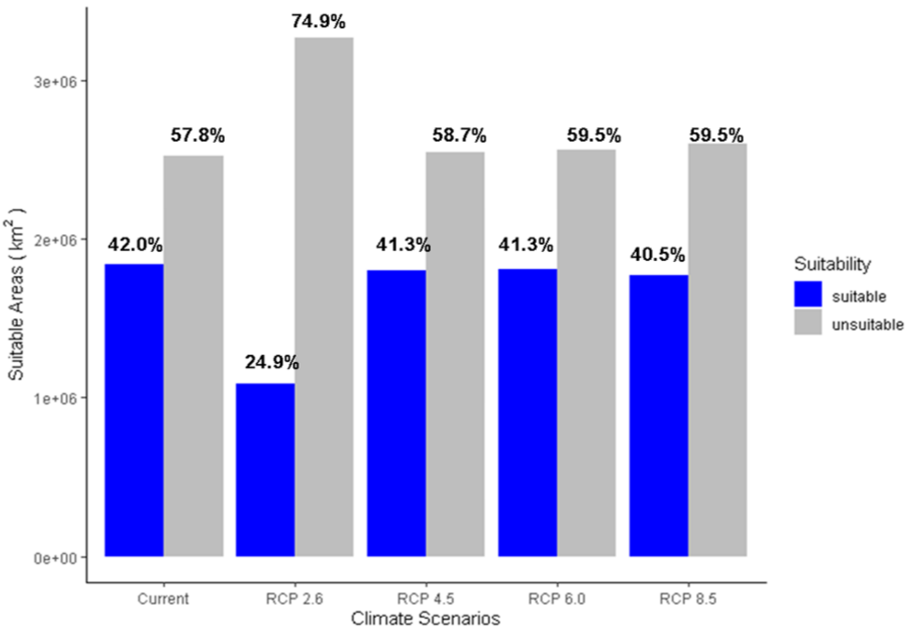


Figure 7. Areas of habitat suitability (km²) of *P. manokwari* in present and future climate scenarios (2070).

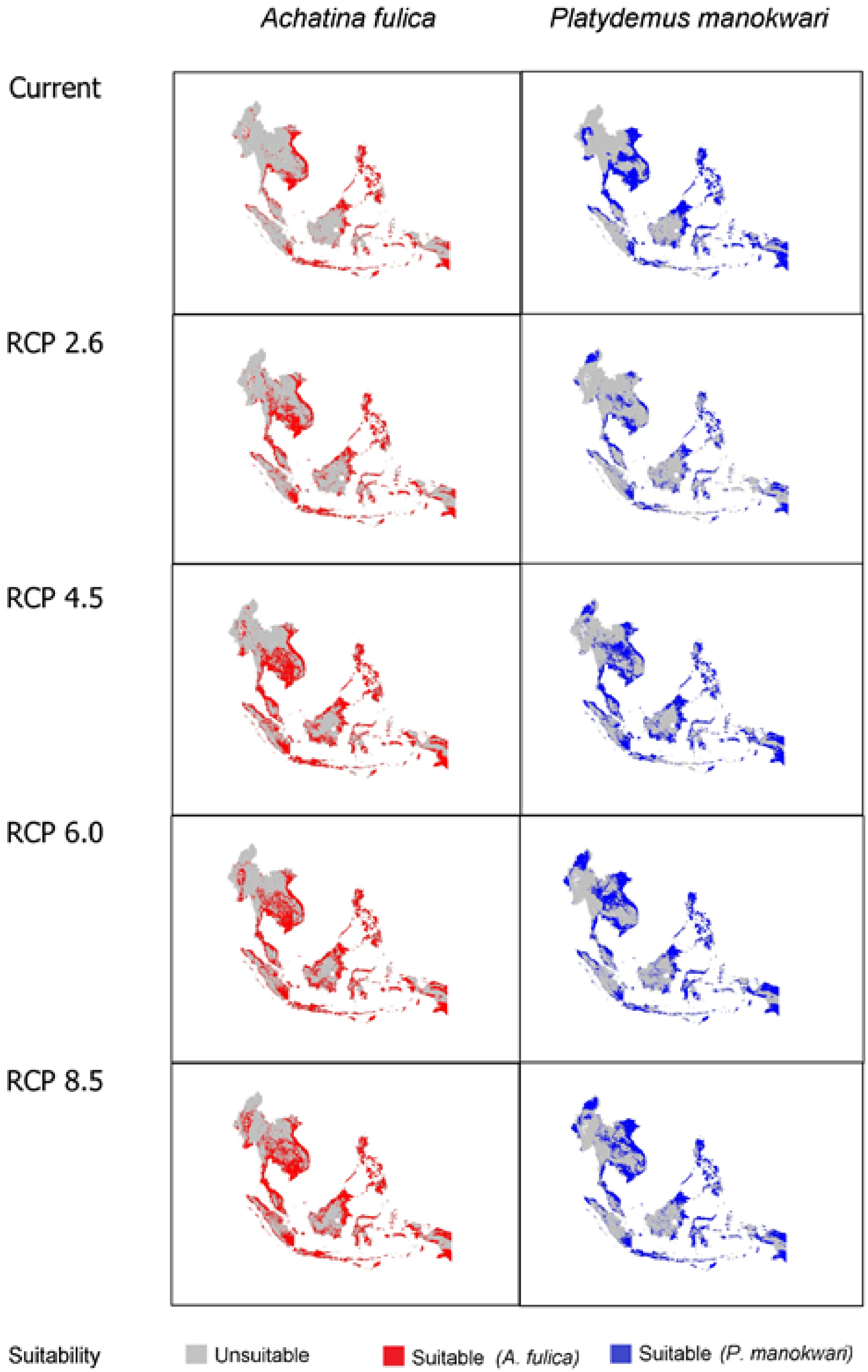


Figure 8. Present and future habitat suitability maps of *A. fulica* and *P. manokwari* in the Southeast Asian region for 2070 in four Representative Common Pathways (RCP) scenarios.

DISCUSSION

Present Suitability

Determining which areas are suitable for a particular species requires consideration of the species' biology, tolerance range, and interaction with other organisms. *A. fulica* and *P. manokwari* are both soil-dwelling organisms that feed on plants and small invertebrates, respectively. Despite the similarity in their environment, these species have different top environmental factors associated with their suitable habitats. *A. fulica's* suitable habitat is primarily associated with soil factors, while *P. manokwari* is associated mainly with climatic ones. Factors such as the volumetric fraction of coarse segments, bulk density of the fine earth fraction, and soil organic carbon are specifically relevant to *A. fulica*. At the same time, the distribution of *P. manokwari* is affected by the annual mean temperature and precipitation of the warmest quarter. Similar findings were also found in the literature, such as how Idohou and Codjia (2013) found the soil type as the main determinant for *A. fulica's* distribution and Gerlach's (2019) findings on the impact of temperature and humidity on *P. manokwari*'s activity.

One possible reason is the presence of *A. fulica's* protective shell, which allows it to conserve moisture in its body. On the other hand, *P. manokwari*'s body is more exposed, making it more sensitive to severe climate changes. However, the differential effect of both climatic and soil variables on these species is poorly documented, making it difficult to ascertain this claim. Soil data is currently not widely used in modeling and experiments alike, especially in studying flatworms. On the other hand, temperature and humidity influence both *A. fulica* and *P. manokwari*'s distribution. For instance, Sarma et al. (2015) found that the temperature mainly influences *A. fulica's* invasion risk. Although they could not account for the soil variables, their results were consistent with the previous studies (Albuquerque et al., 2009; Sharma & Dickens, 2018). *P. manokwari* flatworms, on the other hand, are more susceptible to severe temperature changes than *A. fulica* snails. As shown in Figure 3, *P. manokwari* can only survive temperatures from 25°C to 30°C most of the time, which is consistent with Gerlach's (2019) findings of 24°C to 30°C while *A. fulica* can survive 28°C to 45°C (Sharma & Dickens, 2018).

Aside from the previously mentioned variables, the pH of water in the soil and the mean diurnal range are common in *A. fulica's* and *P. manokwari's* habitat suitability models. Soil acidity influences the bioavailability of nutrients such as nitrogen and phosphorus. Both species generally prefer slightly acidic soils with pH values ranging from 5.0 to 7.0 at 50% probability (Figures 2 and 3). Constant rainfall can lead to high soil acidity, which could explain the currently predicted occurrence of these species in Southeast Asia (Silva et al., 2022), as shown in Figure 6. Rainfall, in turn, directly affects the air's humidity. When rainfall occurs for a long time, the water vapor in the air will increase, which also elevates the moisture content in the soil. *A. fulica's* body size and weight during development increase with increasing humidity (Albuquerque et al., 2009). Similarly, the *P. manokwari* relies on its surroundings' moisture for locomotion (Gerlach, 2019). Preference for areas with high humidity and a specific temperature range could imply that areas with low precipitation and too extreme temperatures have a low risk of invasion.

The mean diurnal range (MDR) is the difference between the highest and lowest temperatures on a single day. This variable is an essential climate change indicator because it correlates with other factors, such as local warming (Libanda et al., 2019). Over the years, there has been a downward trend of MDR, suggesting an unequal increase between the daily maximum and minimum temperatures (Braganza et al., 2004). *A. fulica* and *P. manokwari* are nocturnal organisms; hence, changes in nighttime temperature could disrupt their activity. However, little to no evidence support the relationship between mean diurnal range and nocturnality. In this study, the MDR at the highest logistic probability is 4.0 °C and 4.3 °C for *A. fulica* and *P. manokwari*, respectively (Figures 2 and 3). Reduction in MDR has been observed in the majority of Southeast Asia at approximately 1°C to 2 °C (Hamed et al., 2022). The author noted that the MDR varies in spatial and temporal aspects, highlighting the need to investigate this variable and its effects in localized regions.

In this study, *A. fulica* has lower suitability than *P. manokwari* despite having higher species occurrence data (Figure 5). Other factors could have shaped its distribution aside from the variables applied here, such as the exposure of these snails to anthropogenic activities and possible predation. On the other hand, *P. manokwari* lives in less conspicuous areas, possibly reducing its vulnerability. While the maps can help pinpoint which areas need regular surveillance, further experimental studies are necessary to confirm the findings, specifically on the species' tolerance range. In addition, future research could investigate the dispersal patterns of *A. fulica* and *P. manokwari*. Understanding their movement will help determine the geographic range of the biocontrol agent *P. manokwari* in relation to the invasive *A. fulica* and its spread. The study suggests that drastic changes in the soil and bioclimatic variables could shift species distribution, making it crucial to investigate these dynamics in the context of climate change.

Future Suitability

The suitable areas for *A. fulica* snails were predicted to expand with increasing greenhouse gas concentrations in the year 2070 (Figure 6), implying that these snails may continue to proliferate in the future. Their resistance to extreme temperatures is attributed to their behavioral adaptation. In exceedingly low temperatures, they undergo the process of aestivation, while in arid conditions, these snails hibernate. Their tendency to enter a state of dormancy could help them thrive in the future, regardless of the temperature. These findings, however, were contradicted by Ananthram et al.'s (2022) study. Through niche conservatism, the authors predicted that *A. fulica* would retain its niche in the SSP2 and SSP5 climate scenarios from 2061 to 2080. Unlike RCPs, SSPs (Shared Socioeconomic Pathways) take into account the possible course of action based on different policies toward climate change in their scenarios, providing a more holistic approach Riahi et al. (2017).

The niche of the biocontrol flatworm *P. manokwari* is expected to be maintained in 2070 for RCP 4.5, RCP 6.0, and RCP 8.5. However, for RCP 2.6, a niche contraction is expected to occur. The absence of a trend with increasing RCPs nor the lack of considerable difference between present and future climate scenarios is not expected. A laboratory experiment by Sugiura (2009) found that a temperature of less than 17.1°C limited *P. manokwari*'s feeding activity toward its prey. These findings imply that *P. manokwari*'s invasion risk could increase with elevated temperatures due to climate change. The conflicting results could be due to the varying methods used, specifically the mechanistic nature of Sugiura's (2009) study compared to the correlational approach in this study.

The overall positive response of *A. fulica* and *P. manokwari* to worsening climate scenarios imply their persistence in Southeast Asia, indicating potential future invasions. This finding suggests that biological interactions might be more advantageous for invasive species than their native prey, as higher temperatures could significantly diminish native prey populations. Biodiversity decline poses a negative forecast for the recovery of ecosystems, especially with climate change. While this study projected the average conditions tolerable for these species, future studies could delve into their physiology to validate these findings (Peterson et al., 2015). However, the results may differ depending on whether experiments are conducted in the field or in laboratory settings. Nevertheless, recent literature supports Sugiura's (2009) conclusion on *P. manokwari's* niche expansion. *P. manokwari* is now reported outside New Guinea, such as in Hong Kong, Europe, Japan, and even the French West Indies (Hu et al., 2019; Justine et al., 2021, 2014). Most of these areas have temperate to semi-temperate climates, which is disparate from the humid and tropical characteristics of New Guinea. These studies substantiate the capacity of these flatworms to thrive in new locations.

In Southeast Asia alone, the suitable areas for both *A. fulica* and *P. manokwari* are changing. Presently, *A. fulica* is predicted to thrive in the eastern coasts of the Indo-China peninsula, the central Philippines, and some portions of Brunei and Indonesia. In 2070, however, these areas are expected to change under different climate scenarios. For instance, the suitable areas are expected to shift to the eastern parts of the Indo-China peninsula in RCP 2.6, whereas, in RCP 4.5, movement is predicted towards the northern region. *P. manokwari*'s suitability exhibits these similar dynamics. Some scenarios show a shift toward the northern areas, while others show a shift toward the central area. Hence, there is no single direction in terms of niche shift. While evaluating each species' response to climate change is essential, it is equally important to determine how their interactions could change in the future.

Niche Overlap and Dynamics

The stable interaction between *A. fulica* and *P. manokwari* across time and climate scenarios in Southeast Asia demonstrates the similarity of their niche. The findings of this study imply that these species will continue to coexist in the future, but *P. manokwari* could begin to occupy spaces not occupied by the invasive snail, where they could consume native snail species. Niche unfilling can occur due to several reasons, including differences in environmental tolerance, availability of food, and competition for space. While invasive land snails and flatworms occupy areas with similar environmental conditions, they have different food preferences. *A. fulica* snails consume vegetation and other decaying materials, whereas *P. manokwari* flatworms consume snails, isopods, and other invertebrates. Due to their generalist behavior, *P. manokwari* can survive even without their target invasive species.

One of the early introductions of *P. manokwari* as a biocontrol agent for the invasive *A. fulica* snails was in 1986 for Muniappan et al. (1986) field experiment. The authors deliberately introduced the flatworm in the coconut fields of Bugsuk Islands, Philippines, and found a drastic decrease in *A. fulica* snail populations, implying that the flatworms were effective. However, the authors did not monitor the possible interactions of flatworms with other species in that area. In support of this, Kaneda et al. (1992) determined the suitable conditions for producing more flatworms to regulate the snail population. Later studies would then find that these flatworms also consume native land snails, as observed in the Osagawara Islands, Japan – marking them as another invasive species (Sugiura, 2010). Despite initial claims of being an effective biocontrol agent for *A. fulica*, *P. manokwari* is now classified as one of the world's most invasive species. In their review, Gerlach et al. (2021) highlight these flatworm species as ineffective, urging the community to terminate their introduction to new areas.

To be effective biocontrol agents, biocontrol candidates must establish their populations in the same environmental conditions as their target invasive species. One aspect to consider is the biocontrol agent's role as a consumer. For example, in their native ecosystems, *A. fulica* functions as a primary consumer and detritivore, aiding in the cycle of nutrients, while *P. manokwari* acts as a secondary consumer. These species could still maintain their ecological roles when introduced to new areas, potentially disrupting established biological interactions. When the local and introduced species have the same functional role in one community, competition could arise threatening the fitness of the involved species. It is important to emphasize that biocontrol agents are also introduced, which means they are subject to the same constraints as invasive species. In this study, the niche overlap and dynamics were evaluated based only on the species occurrence data. Future researchers could investigate biological interactions such as inter- and intra-specific competition at a finer scale using stable isotopes (Rosengren & Magnell, 2024).

Future Directions

This study mainly focuses on the interaction of organisms on a macroecological scale. To support the findings of this study, future research could explore the mechanistic aspect of the species' relationship by evaluating the biological and physicochemical parameters to a finer extent. Moreover, since anthropogenic activities highly influence dispersal and species distribution, their impact on niche shift could be further investigated. Future researchers can also model the interaction between invasive species and their potential hosts at different trophic levels to fully characterize the invasion risk. In soil communities, *A. fulica* snails are primary consumers, while *P. manokwari* flatworms are notorious predators. Both species are intermediate hosts of Angiostrongylus cantonensis. Investigating the impact on both lower and higher trophic levels will aid in an integrative understanding of the role of these species in biodiversity loss.

Future research on niche dynamics should focus on identifying the specific direction of the niche shift by evaluating changes in the centroid of the population density. Through this method, conservation efforts can pinpoint specific places for control. While biocontrol through the introduction of natural enemies in invaded areas is promising, it carries risks, such as the potential extinction of native species. Inducing the local extinction of these invasive species could complement effective biological control strategies (Sharma & Dickens, 2018; Gerlach, 2019). For instance, determining the species' tolerance limits to temperature and humidity could simulate a hostile environment, reducing their probability of survival and aiding in their control without introducing potential invasive species.

CONCLUSIONS

The suitable habitats for the invasive *A. fulica* snails in the Southeast Asian region are predicted to expand in 2070. On the other hand, the ratio of suitable to unsuitable areas for *P. manokwari* will remain the same, except in RCP 2.6. The acidity of water in the soil (phh2o) and the mean diurnal range (bio2) are the two common variables affecting both species. Bioclimatic factors such as temperature and precipitation are found to mainly influence *P. manokwari's* distribution, while soil variables primarily affect *A. fulica*. Furthermore, the niche overlap between the two species is predicted to be stable in the future. However, there may be areas suitable only for *P. manokwari* and not for *A. fulica*. This could lead to *P. manokwari* flatworms preying on native snail species. Therefore, despite their similar niches, *P. manokwari* flatworms may not be an appropriate biocontrol agent in the long term. It is critical to assess the compatibility of a biocontrol candidate not only with the target species but also with other species within the community. Understanding how the niches of these species contract or expand will help determine areas vulnerable to biodiversity loss and potential outbreaks of infectious diseases, particularly in regions where these species serve as hosts for parasitic nematodes.

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THE EFFECT OF ORGANIC LEACHATE AND RICE WASHING WATER ON THE COMPOSTING TIME OF ORGANIC WASTE

Husnawati Yahya[1*], Arief Rahman and Rezeki Win Kuara[1]

[1]Environmental Engineering Study Program, Faculty of Science and Engineering, State Islamic University of Ar-Raniry, Banda Aceh, 23111, Indonesia

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***Corresponding author:**
husna.83@ar-raniry.ac.id

ABSTRACT

Waste is an integral part of human life. The increase in population is one factor that increases the waste generated. The more the population, the more activities are carried out. Organic leachate and rice washing water are environmentally friendly compost activators and are easily obtained from daily activities. Composting is a waste management strategy. Composting is an organic method through the activity of microorganisms to decompose natural materials into materials, including humus. The purpose of this study was to determine the moisture content, temperature, pH, C/N ratio, and macro nutrients during the composting process. The physical parameters of compost from the three activator concentrations showed that the treatment of P3 (with the addition of leachate formulation 20 ml/kg and rice washing water 15 ml/kg) and P2 (with the formulation adding 20 ml/kg leachate and 10 ml rice washing water), the physical compost were brownish black in color, fine textured and smelled of soil on the 22nd day. The P3 treatment has met the quality criteria of compost according to SNI 19-7030-2004, the moisture content (30%), temperature (27 °C), pH (6,8), C-Organic (32,9%), Nitrogen (1,42%), Phosphor (0,65%) and Potassium (1,63%). For the C/N ratio, the result of all treatments did not meet the quality criteria of compost according to SNI 19-7030-2004. The C/N ratio of all treatments showed that it is still above the maximum standard that has been set at 20.

Keywords:
Organic waste, leachate organic, rice washing water, composting time, compost quality standard

INTRODUCTION

Composting is one of the waste processing strategies and is an organic method through the activities of internal microorganisms breaking down natural materials into materials, including humus (Sutanto, 2002). In the composting cycle, microorganisms can emerge from the rotting waste. The carbohydrate content in rice washing water can be used as a source of food supply for microorganisms. Meanwhile, leachate is added to add microorganisms to the composting cycle (Suwahyono, 2014).

Naturally, organic materials occur as a result of weathering compost, but it takes a long time, about half to 12 months (Suryati, 2014). The composting process can be accelerated with the help an activator (Suwahyono, 2014). In this research, two types of activators were used, there were leachate and rice-washing water. Leachate is water in the form of water waste technology that dissolves several existing compounds so that it contains a lot of pollutants, especially organic materials (Darmasetiawan, 2004). However, leachate has the potential to be used as an organic fertilizer because it carries many organic substances, which include nitrates, minerals, and organisms (Ali, 2011). This very high content of organic substances will increase the activity of microorganisms in degrading organic waste (Rosariawari, 2012).

MATERIALS AND METHODS

The tools used in this research were a Soil Tester, Thermometer, pH meter, Measuring Cup, Scales, Plastic waste basket 4 pieces, gloves, and mask. Meanwhile, the materials used include rubbish vegetables and dry leaves with a comparison of the composition of vegetable waste: dry leaves = 1 kg: 0.8 kg (based on calculations by the Ministry of Agriculture and Food, 1998 to obtain a C/N ratio of 25), then wood dust, washing water rice, and leachate. This research had been used the Takakura Method. Composting using the Takakura method was applied in an aerobic process. In experiments, there was no replication. This experiment consisted of four treatments, including control (without adding leachate and rice washing water). For treatment 1 (P1) with the formulation adding 20 ml/kg leachate and rice washing water 5 ml rice, treatment 2 (P2) with the formulation adding 20 ml/kg leachate and 10 ml rice washing water, treatment 3 (P3) with the addition of leachate formulation 20 ml/kg and rice washing water 15 ml/kg (Dewi et al., 2016). The moisture content, pH, and temperature were determined daily for data analysis. Macro nutrients such as C-organics, Nitrogen, Phosphor, Potassium, and C/N ratio were determined after 28 days of composting.

RESULTS AND DISCUSSION

Times of Composting

Composting time is the length of time the organic waste decomposes and changes its texture to soil (Nurullita & Budiyono, 2012). The length of the composting process for each treatment can be seen in Table 1.

Duration/composting time for each of these concentrations is 25 days (P1), 23 days (P2), and 22 days (P3), with physical compost smooth textured. By physical observation, the organic waste began to degrade on day 14. The colour of the waste began to turn blackish, the texture was fine, and it began to smell like soil. Meanwhile, until the 28th day, the texture was still rough in the control container, the leaves and grass were still intact, and the composting process stopped. These results are in accordance with research from Wiryanti (2014), who stated that, fermented organic waste without activators arrived on the 30th day, and the texture is still hard like a leaf.

In this study, the fastest composting process occurred in P3 treatment (by adding leachate formulation 20 ml/kg and rice washing water 15 ml/kg) in organic waste (dried vegetables and leaves) as much as 1: 0.8 kg. This treatment can convert organic waste into compost within 22 days.

This is thought to be because the activator in P3 treatment is more contains many microorganisms that are capable of degrading materials organic. These results are in accordance with the results found by Sriharti and Salim (2010), which state that activators contain microorganisms that can work effectively in the fermentation of organic materials, microorganisms These are photosynthetic bacteria, lactic acid bacteria (*Lactobacillus* sp), yeast, *Actinomycetes* and other types of microorganisms.

During the composting process of organic waste (dried vegetables and leaves), the addition of different organic leachate activators and rice washing water in P1(20:5), P2 (20:10), P3 (20:15) can shorten composting time to 22 days (P1), 23 days (P2) and 25 days (P3). Meanwhile, in the P0 (without adding the organic leachate and rice washing water) container until the 30th day no decomposition process occurs. In accordance with research by Dewi et al. (2016) who stated that the addition of activators was proven to be able to decompose organic ingredients effectively, so that the ripening process takes place quickly.

By data analysis, some parameters of SNI 19-7030-2004, such as pH, temperature, moisture content, and macro nutrients (C-organic, Nitrogen, Phosphor, and Potassium), are in accordance with SNI standards. The temperature reached 40-48 °C (maximum temperature) from the first day until the sixth day. This is due to the active microorganisms that decompose organic matter in waste. Parameters of pH and moisture content are also according to SNI standards. Bacterial activity

Table 1. Data Analysis on Composting Process Duration/Time from Varying Concentrations Activators and Controls

Treatments	Composting Time	The average of			Macro nutrients				
		Temperature (°C)	Moisture content (%)	pH	C-organics (%)	Nitrogen (%)	Phosphor (%)	Potassium (%)	C/N ratio
P0	More than 30 days	27 - 38	30 - 50	6 - 6,9	36,59	1,12	0,32	1,48	32,66
P1	25 days	27 - 38	30 - 50	6,4 - 6,9	35,49	1,29	0,36	1,57	27,51
P2	23 days	27 - 38	30 - 50	6,4 - 6,9	32,86	1,59	0,44	1,60	21,61
P3	22 days	27 - 38	30 - 50	6,2 - 6,9	32,09	1,42	0,65	1,63	22,59
SNI 19-7030-2004		groundwater temperature	Minimum: - Maximum: 50	Minimum: 6,80 Maximum: 7,49	Minimum: 9,80 Maximum: 32	Minimum: 0,40 Maximum: -	Minimum: 0,10 Maximum: -	Minimum: 0,20 Maximum: -	Minimum: 10 Maximum: 20

in the P3 treatment showed that there was good decomposition of organic compounds characterized by decreasing C levels during composting. From Table 1, the treatment of P2 and P3 showed an increase in nitrogen value, P2 (1,59%) and P3 (1,42%). The higher the nitrogen content, the lower the C/N ratio. This research showed that the C/N ratio from all treatments is still above 20. A high C/N ratio in compost can be assumed that decomposing microorganisms are still active in degrading the compost. Biologically, the decomposition process continues until the C/N ratio of the soil is reached (Mirawati & Winarsih, 2019).

Based on SNI 19-7030-2004, to improve the quality of compost can be done by: making granules, drying and sieving. In this study, the drying process was carried out on day 22 to day 25. Sifting was done on the 28th day. We can add the activator for shorten composting time.

CONCLUSION

Organic leachate and rice washing water as activators speeds up composting time and improves compost quality compared to controls without the addition of activator. Duration/composting time for each of these concentrations are 25 days (P1), 23 days (P2) and 22 days (P3), with physical compost smooth textured. The addition of leachate and rice washing water also indicates compost quality better. The P3 treatment has met the quality criteria of compost according to SNI 19-7030-2004, the moisture content (30%), temperature (27°C), pH (6,8), C-Organic (32,9%), Nitrogen (1,42%), Phosphor (0,65%) and Potassium (1,63%). For the C/N ratio, the result of all treatments did not meet the quality criteria of compost according to SNI 19-7030-2004.

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CRAB DIVERSITY IN MANDALIKA AND THEIR COMMERCIAL VALUE

Dewi Citra Murniati[1*], Tri Arfianti[1]

[1]Research Center for Biosystematics and Evolution - National Research and Innovation Agency, Cibinong, West Java, Indonesia

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***Corresponding author:**
dewi015@brin.go.id

ABSTRACT

West Nusa Tenggara, Indonesia, encompasses the western part of the Lesser Sunda Islands with a long coastline that supports a rich biodiversity of coastal biota. Brachyuran crabs constitute one of the most diverse invertebrate groups in the coastal ecosystem. This research aimed to record the biodiversity of brachyuran crabs and their commercial value in the Special Economic Zone (SEZ) of Mandalika in Central Lombok, Indonesia. Data were collected by hand, scoop, and trap using the random sampling method. In the current study, we have compiled, for the first time, a checklist of 402 individual brachyuran crabs belonging to 22 genera, 13 families, and 35 species from Mandalika. The present study revealed that Mandalika has a comparable number of species to those in previous studies from other coastal areas on Lombok Island. The commercial value of the crabs is in line with their edibility. The family Ocypodidae comprised 43% of all the brachyuran crabs reported from Mandalika. The findings emphasise the importance of their habitat in Mandalika and the potency of brachyuran crabs to support government food security programs.

Keywords:
Biodiversity, Crustacea, Food Security, Coastal Area, Taxonomy

INTRODUCTION

Mandalika Special Economic Zone (SEZ) is between two of Indonesia's most well-known tourist destinations: Bali and Komodo Islands. Due to its strategic location on a tourist route, the West Nusa Tenggara Government has selected Mandalika as a priority zone to support the regional economy and ecotourism since 2014 (Adam, 2019). Following that, the local government's rapid infrastructure development has led to massive area conversion, such as Pertamina International Circuit Mandalika, which covers 1,035.67 hectares of area (Birkic et al., 2019; Nisak & Ristawati, 2023).

The area conversion in Mandalika may contribute to high economic growth in Lombok Island. However, there are major problems that result from converting land area for infrastructure, such as decreasing agricultural production, air pollution, and soil erosion (Harini et al., 2017; Wirosoedarmo et al., 2017; Vanaker et al., 2019). Therefore, continually assessing the environment and its biota is necessary to prevent further negative impacts. Mandalika is very famous for its developed coastal areas. Hence, it is important to monitor its coastal environment. One of the fauna that inhabit the coastal area is the brachyuran crab.

Renowned for its pure and magnificent nature scene for years, Mandalika SEZ features a long beach with clear water and exotic white sand that supports high biodiversity (Ardhiati et al., 2021), including brachyuran crabs. Brachyuran crab is a member of the crustaceans, commonly known as a protein source with high economic value for society. Portunidae is a family of brachyuran crabs, and its members are recognized for commercial importance, such as the genus of *Charybdis, Portunus, Scylla,* and *Thalamitha*. Besides those genera, some members have important potencies, such as edible crab, but are not yet popular among locals (Ng et al., 2008; Stevcic, 2005).

Previous studies have recorded the brachyuran crab species from several areas on Lombok Island. Anggorowati (2014) have recorded 66 species from West Lombok, Murniati (2015) have recorded 10 species of deposit-feeder crab from West and East Lombok, and Murniati (2017) have recorded 35 species of brachyuran crab from eastern part of Lombok Island. However, up to now, information on the brachyuran crab diversity in Mandalika and its commercial value remains limited. Thus, this study aims to present the first species list of brachyuran crabs from Mandalika SEZ and their commercial value. Meanwhile, the sustainable biodiversity observation in the conversion area benefits data that help stakeholders decide on the conservation efforts (Mohanty et al., 2019).

MATERIALS AND METHODS

Samples were collected on 7–13 March 2018 from six sites, i.e., Muara Sungai Tebelo (MST), Mangrove Tanjung Aan (MTA-1), Mangrove Tanjung Aan 2 (MTA-2), Pantai Benjon (PB), Pantai Seger (PS), and Bukit Batu Payung (BBP) (Fig. 1). The brachyuran crabs were collected by hand, using small scoop and trap with bait with random sampling method. Species identification using Crane (1975), Ng (1998), and Rahayu & Setyadi (2009). Species and the number of specimens collected from each sampling site were recorded and deposited at the Museum Zoologicum Bogoriense (MZB), Cibinong, West Java. The edibility of each species and whether they are traded in the local market was recorded based on the local information, Ng (1998), and regulation number 12 of 2020 concerning the management of lobsters, and crabs in the territory of the Republic of Indonesia (The Minister of Maritime Affairs and Fisheries of the Republic of Indonesia, 2020).

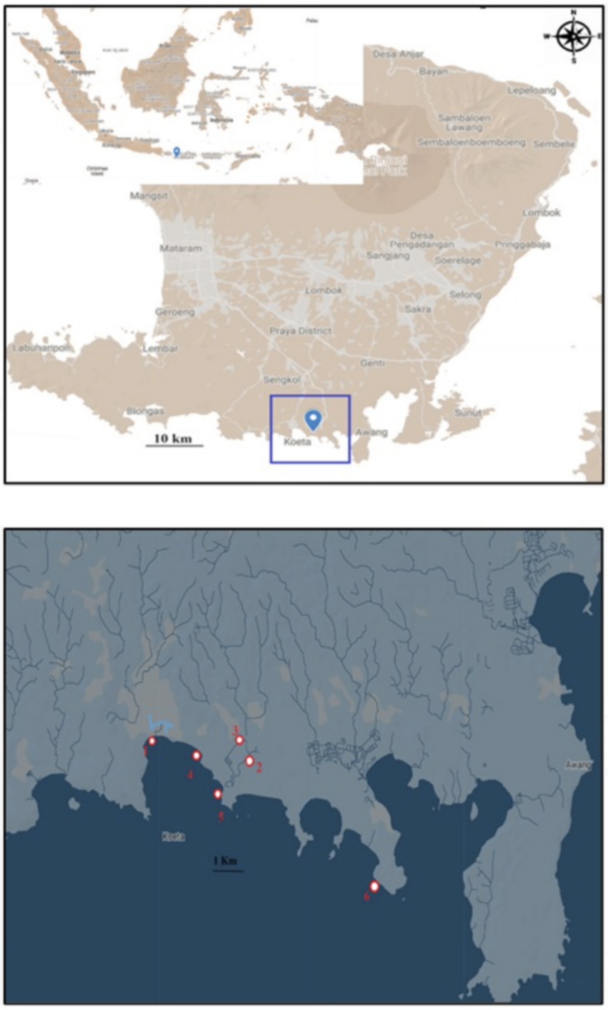

Figure 1. The map showing six study sites of the brachyuran crabs in Mandalika Tourism Special Economic Zone (SEZ), Lombok, West Nusa Tenggara

RESULTS AND DISCUSSIONS

This research revealed 402 brachyuran crabs representing 13 families, 22 genera, 35 species, and seven specimens identified to genus level at six sites (Table 1). Twelve species were recorded from MST, five species from MTA-1, nine species from MTA-2, fifteen species from BBP, eight species from PB, and nine species from PS. Species of Ocypodidae was recorded from five out of six sampling sites. Meanwhile, Grapsidae and Sesarmidae were recorded from four sampling sites.

Within the recorded species, nine species are edible, i.e., *Cardisoma carnifex, Episesarma versicolor, Grapsus albolineatus, Grapsus tenuicrustatus, Myomenippe hardwicki, Ocypode ceratophthalma, Portunus pelagicus, Thalamita crenata, Thalamita* sp1., and *Varuna litterata* (Fig. 2) (Ng, 1998). Locals commonly known as three species of "rajungan" are *Portunus pelagicus, Thalamita crenata*, and *Thalamita* sp1., while other species are known as "yuyu". *Cardisoma carnifex* has a large body size that reaches 15 cm carapace width. In other regions, such as Papua, locals consume this crab daily (Murniati, 2023). However, on Lombok Island, the locals consumed this crab only if they found it in an abundant population, especially during the rainy season. This result shows that despite the small record of brachyuran crabs, Mandalika SEZ has a high potency of valuable protein resources. Further exploration for monitoring purposes would reveal more data than that presented here.

Brachyuran crabs are helpful not only as food resources but also as indicators for environmental assessment. For example is *Tmethypocoelis* sp1 which recorded only in MTA-2. This crab has small body size and is known to be hardly adapted to high-polluted environments (Fig. 3). *Tmethypocoelis* indicates that the area has low or zero contamination of toxic pollutants (Dutrieux, 1992).

Figure 4 shows the representative record for the diversity of brachyuran crabs around Mandalika SEZ. Ocypodidae took the highest percentage of the occurrence since this group was readily observed in their habitat. This family is active during the day at low tide in open areas and has a slow movement; thus, it is relatively easy to observe and catch (Hughes, 1966). Meanwhile, other families, such as Calappidae, Menippidae, and Oziidae, usually hide between rocks on rocky beaches. Most of these brachyuran crabs were collected by hand.

The specific fishing method for each group or family is essential to gain more brachyuran crab species. Therefore, further interviews with the locals, habitat observation, literature study, and experiments with different tools are necessary. For example, the fisherman uses a gillnet or mini trawl for fishing *Portunus* (Ihsan et al., 2014), a lift net with bait for fishing *Scylla* (Sahat et al., 2015), and mousetrap for collecting *Cardisoma* (Takahashi & Nishida, 2018).

Table 1. Checklist of the brachyuran crabs from Mandalika, West Nusa Tenggara, including their edibility information and commercial value.

Taxa	Study Sites						Total	Edibility	Commercial value (traded)
	MST	MTA 1	MTA 2	BBP	PB	PS			
Gecarcinidae									
1. *Cardisoma carnifex*	3						3	V	-
Grapsidae									
2. *Metopograpsus quadridentatus*	7						7	-	-
3. *Grapsus albolineatus*				3	4	3	10	-	-
4. *Grapsus tenuicrustatus*				5	2		7	-	-
5. *Pseudograpsus albus*						12	12	-	-
Ocypodidae									
6. *Austruca annulipes*	4		34				38	-	-
7. *Austruca perplexa*	23						23	-	-
8. *Ocypode ceratophthalma*	13				5	3	21	V	-
9. *Tubuca coarctata*	4		1				5	-	-
10. *Tubuca demani*	5	14	30				49	-	-
11. *Tubuca dussumieri*	8		9				17	-	-
12. *Tubuca forcipata*		4					4	-	-
13. *Tubuca* sp1.		11	6				17	-	-
Varunidae									
14. *Metaplax distinctus*	15						15	-	-
15. *Varuna litterata*	6						6	V	-

Taxa	Study Sites						Total	Edibility	Commercial value (traded)
	MST	MTA 1	MTA 2	BBP	PB	PS			
Sesarmidae									
16. *Episesarma versicolor*			6				6	V	-
17. *Parasesarma* sp1	8		20				28	-	-
18. *Parasesarma* sp2	8	5	11				24	-	-
19. *Neosermatium* sp1		2					2	-	-
Dotillidae									
20. *Tmethypocoelis* sp1.			34				34	-	-
Calappidae									
21. *Calappa hepatica*				2			2	-	-
Carpiliidae									
22. *Carpilius convexus*				3			3	-	-
Eriphiidae									
23. *Eriphia sebana*				1		1	2	-	-
Grapsidae									
24. *Grapsus albolineatus*				3	4	3	10	V	-
25. *Grapsus tenuicrustatus*				5	2		7	V	-
26. *Pseudograpsus albus*						12	12		-
Menippidae									
27. *Myomenippe hardwicki*				1			1	V	-
28. *Myomenippe* sp1.				2			2	-	-
Oziidae									
29. *Epixanthus dentatus*				5			5	-	-
30. *Ozius guttatus*				3			3	-	-
31. *Ozius tuberculosus*				2			2	-	-
32. *Ozius truncatus*						4	4	-	-
Portunidae									
33. *Portunus pelagicus*				4	1	3	8	V	V
34. *Thalamita crenata*				1	2	2	5	V	V
35. *Thalamita* sp1.				1	7		8	V	V
Total	**104**	**36**	**151**	**41**	**27**	**43**	**402**		

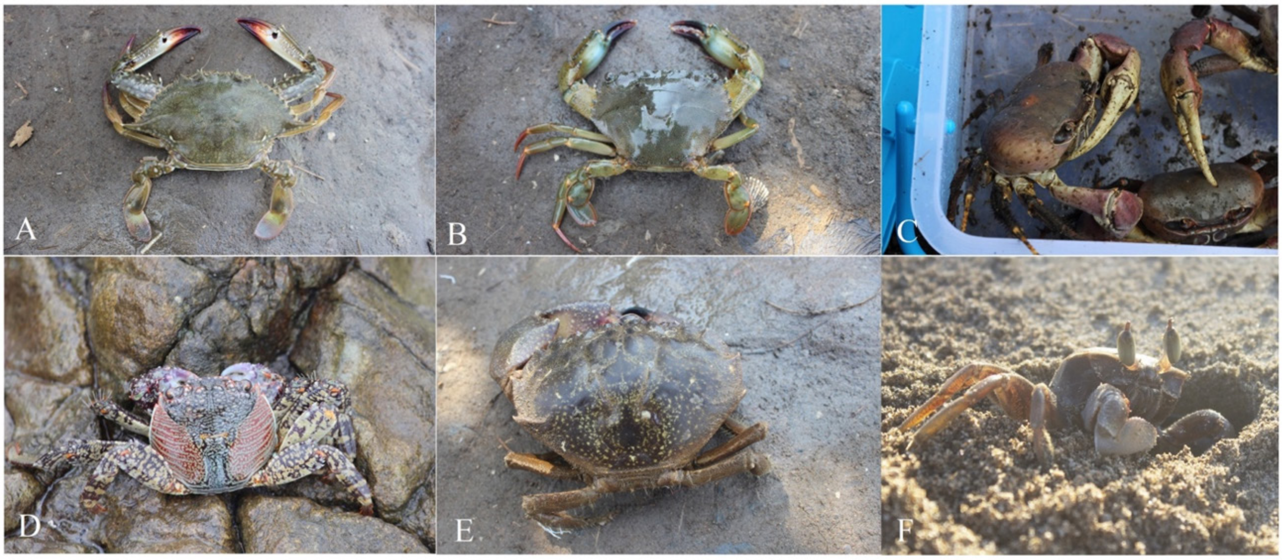

Figure 2. Representative of edible brachyuran crabs from Mandalika, Central Lombok, West Nusa Tenggara. *A. Portunus pelagicus, B. Thalamita crenata, C. Cardisoma carnifex, D. Grapsus albolineatus, E. Myomenippe hardwicki, F. Ocypode ceratophthalma*.

Figure 3. Male *Tmethypocoelis* sp1 collected from MTA-2, Mandalika, Central Lombok, West Nusa Tenggara.

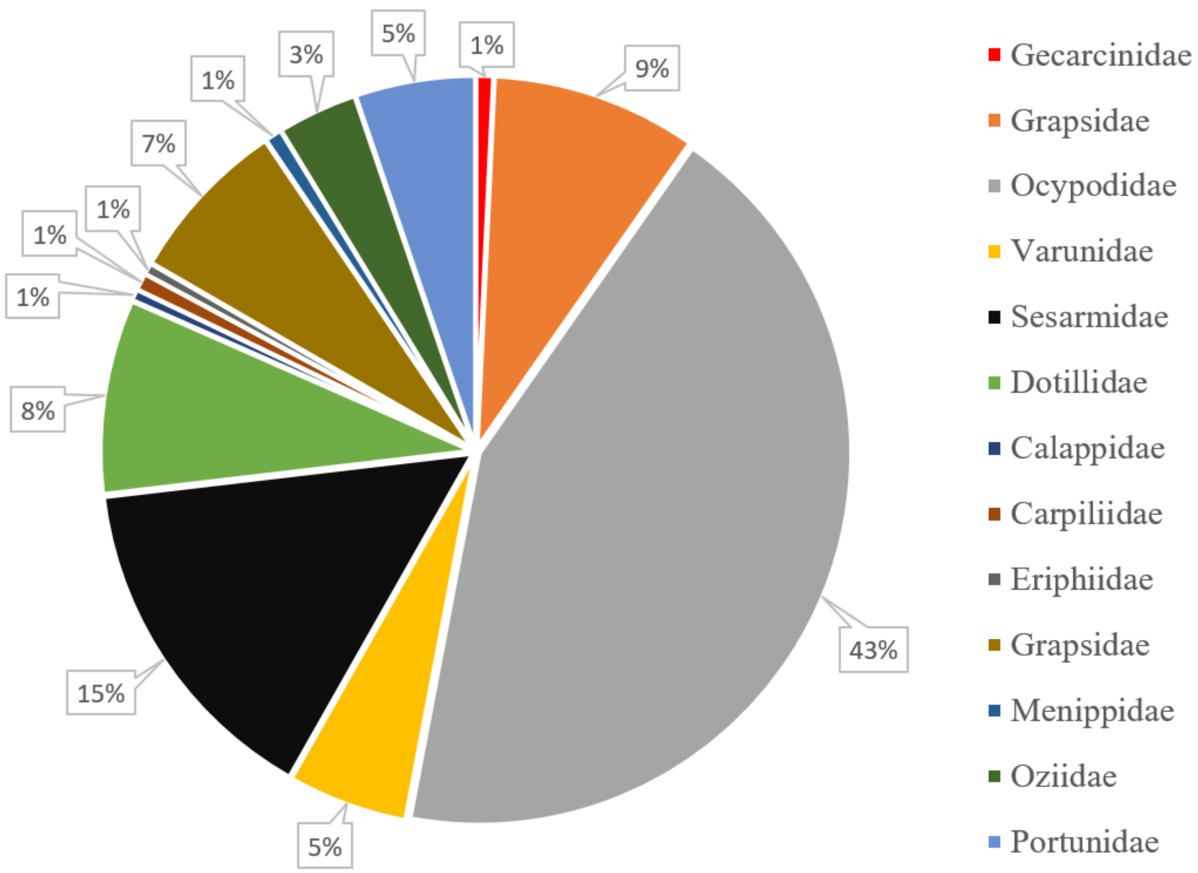

Figure 4. The diversity of the brachyuran crab's family at Mandalika (in percentage).

CONCLUSIONS

The brachyuran crab in Mandalika is mostly a member of the Ocypodidae, and almost half of all brachyuran crabs reported in this study are members of this family. The present study also revealed that there are 35 species of brachyuran crabs in Mandalika, nine of which are edible. The crabs' commercial value aligns with their edibility; thus, protecting their habitat is essential to supporting government food security programs. We recommend doing further research with time series sampling to better understand the stock pattern in nature.

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SPORE PRODUCTION AND INOCULUM FORMULATION OF *CLAROIDEOGLOMUS ETUNICATUM* AND ITS APPLICATION IN MAIZE (*Zea mays*)

Risa Rosita[1]*, Deden Dewantara Eris[2], Aslan Aslan[3], Zulhamsyah Imran[1]

[1]Environmental Technology and Security Section, Science Innovation and Technology Department, SEAMEO BIOTROP, Indonesia

[2]Plant Protection Research Group, Indonesian Oil Palm Research Institute (IOPRI), Indonesia

[3]PT Amman Mineral Nusa Tenggara, West Nusa Tenggara, Indonesia

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***Corresponding author:**
risa@biotrop.org

ABSTRACT

Recently, the demand for AMF (Arbuscular Mycorrhiza Fungi) fertilizer for agriculture and forestry plantations in Indonesia has increased significantly. Meanwhile, the unavailability of an applicable AMF inoculum that's easy to use and inexpensive is one of the constraints on AMF application as a fertilizer in the field. It indicates that the best-formulated inoculum must be found. The study aimed to produce AMF spores using conventional and hydroponic systems, formulate the best AMF inoculum, and measure the growth response in maize (*Zea mays*) after applying the formulated inoculum. At the formulation stage, the research was designed using a Completely Randomized Design (CRD) with three factors: the type of carrier material, temperature, and addition of AMF inoculum. Data were analyzed using SAS software version 9 and further tested using Duncan's Multiple Range Test (DMRT) at 5% alpha (α) level. The results showed that the conventional pot culture technique effectively increases AMF spore density, and the NFT hydroponic technique effectively increases root colonization percentage. The E2 treatment with *Claroideoglomus etunicatum* in pot culture generated the highest spore density of 350 ± 6.97 spores per 10 g of zeolite ($P<0.05$) compared to other treatments and control. Meanwhile, the hydroponic technique had the highest AMF percentage colonization with the E2 treatment at 98% ± 2.66% ($P<0.05$). Analysis of variance in the formulation stage showed the formulated AMF inoculum (TZ60F1, TZ60F2, TZ40F1, TZ40F2) can increase the growth of *Zea mays* compared to not giving formulated AMF inoculum (TK60F1, TK60F2, TK40F2, TK). Compared to other treatments and controls, the F1 carrier media (sodium alginate and 10% *Aloe vera* extract), which makes up the formulated AMF inoculum treated at a temperature of 60 °C (TZ60F1), had a very significant influence on all growth parameters. The most significant plant height was 120 ± 1.70 cm, the number of leaves was 15 ± 0.84, and the largest plant dry biomass was 26.3 ± 2.46 g. Carrier materials in sodium alginate and *Aloe vera* helped protect *C. etunicatum* spores well; even though they were treated with high temperatures of up to 60°C, AMF could still grow and function well.

Keywords:
Colonization, nutrient film technique, pot culture system, spore density

INTRODUCTION

Population growth needs sufficient food production to meet food security and combat hunger and poverty. Berners-Lee et al. (2018) stated that crop production is only sufficient to provide food for a projected global population of 9.7 billion in 2050. However, there are some obstacles to increasing food crop production due to the increase in fertilizer costs in recent years. On the other hand, the effects of dependence on chemical fertilizers and pesticides are known to have caused a decline in human health, disruption of ecosystem functions, and environmental degradation (Proia et al., 2013; Rani et al., 2021; Richardson et al., 2014; Rajput et al., 2020;).

Not all plants can grow in extreme weather and adapt to climate change. Therefore, plants need microbes to survive in bad weather and unpredictable climate change. Regarding this problem, a study about exploring and optimizing the beneficial interactions among microbes and plants is needed to support food production without affecting the relationship between humans and their environment.

Arbuscular Mycorrhiza Fungi (AMF) is one of the soil microbes that play an important role in promoting plant growth. AMF functions as a biological fertilizer because of its ability to assist plants in absorbing nutrients through the mechanism of root colonization. The mechanism appears through the formation of intercellular, intracellular, arbuscular, and vesicle hyphae in the roots of host plants. AMF require a host plant in its life cycle through a colonization process in its roots intracellularly, beginning with the pre-symbiotic to the symbiotic phase (Souza, 2015). These functional structures may help plants to grow nicely. The interaction of AMF and host plants is determined primarily by the suitability of the symbiosis between the fungus and host plant in the root system. AMF provide 80% inorganic nutrition for land-living plants (Begum et al., 2019). On the other side, they gain up to 20% monosaccharides from CO_2 fixation by plants.

Previous studies have been done by Rosita (2021) and Wulandari et al. (2022), which show that other roles of mycorrhizae improve post-mining soil fertility and provide the essential compounds that plants need. Srivastava et al. (2017) report that AMF as a biological fertilizer increases plant growth by accelerating nutrient uptake from the soil, especially inaccessible nutrients such as Phosphate (P) and Nitrogen (N). In addition to absorbing mineral nutrition, AMF maintains root hydraulic conductivity and enhances plant net photosynthetic capacity and stomatal conductance. Glomus fasciculatum increases photosynthesis, plant productivity, and salt stress tolerance (Ebrahim & Saleem, 2017). Two species of AMF that have optimal effects on plant growth and percentage of mycorrhizal infection are *G. facsiculatum* and *G. etunicatum* (Rahayu, 2014). AMF phylogeny defined *G. etunicatum* as the synonym of *Claroideoglomus etunicatum* (Schüßler & Walker, 2010).

AMF production technology has been developed since the role of AMF for plants is known. The production of AMF inoculum requires a system that allows the production of AMF in large quantities, limited space, and effective management. Aryanto et al. (2018) stated that the interaction of the NFT system and AB Mix nutrition with the host plant Pueraria javanica shows the best biomass and spore production, significantly (P<0.05) achieving the highest shoot dry matter, dry root matter, and spores with a percentage of root colonization more than 96%. Rosita et al. (2020) define refers to microscopic observations that have been done on AMF inoculated treatment using Signal grass (*Brachiaria decumbens*) as a host plant, showing AMF colonization reached 55% and spore density amount of 252 % per 10 g media. In contrast, this doesn't occur with non-inoculated treatment.

Nowadays, AMF's inoculum demand for agriculture and forestry plantations in Indonesia is very high. The unavailability of inoculums for application is a major constraint on the use of AMF as a biological fertilizer in the field. Inoculum formulation can be produced in various ways, including tablet formulation, the most inexpensive and effective AMF formulation for field-scale applications. One of the primary standards that must be found in inoculum formulation is inoculum viability, as indicated by the ability of the fungus to colonize host roots and form colonizing structures such as spores, internal hyphae, external hyphae, arbuscles, and vesicles. Using inoculums without a formula usually encounters various obstacles, including the rapid decline in inoculum quality and difficulties in storage, distribution, and application in the field. The objectives of the research were (1) to study AMF spore production techniques using conventional and hydroponic systems and (2) to study the effect of formulated inoculum on the growth of maize (*Zea mays*).

MATERIALS AND METHODS

Area study. The experiments were carried out at the Southeast Asian Ministers of Education Organization Biology Tropical (SEAMEO BIOTROP) greenhouse and Biosystem and Landscape Management (BLM) laboratory, which is located in Bogor, Indonesia, with a latitude of -6.635486261817926 and a longitude of 106.82536873959347.

Preparation of the planting medium. Zeolite was used as a planting medium. The first step was to wash the zeolites thoroughly with running water to remove any dust that might be attached to the surface. Once the washing water ran clear, the zeolite was placed in a holding container to dry in the sun until completely dry. Afterward, the zeolite is carefully weighed and prepared to be placed into a heat-resistant plastic bag. This bag then would be sterilized using an autoclave for 120 minutes at a temperature of 121°C. Two techniques were utilized to produce spores: the conventional pot culture (CVN) and the hydroponic using Nutrient Film Techniques (NFT). Concerning the spore production stage, (1) *Zea mays*, (2) Sorghum bicolour, and (3) *Pueraria javanica* were chosen as host plants. AMF spores of *C. etunicatum* (E) and *G. fasciculatum* (F) were used. Those AMF inocula were obtained from the Biosystem and Landscape Management Laboratory of SEAMEO BIOTROP, with the code numbers BLM_MGL1 and BLM_MGL2. A total of 5 AMF spores were inoculated into each pot in those systems.

Preparation of conventional pot culture (CVN) technique. In establishing the conventional technique using a pot culture system, 5 spores of AMF were inoculated onto the root surfaces and incubated for 4 weeks. The roots not inoculated by AMF were used as the control (K). The inoculation method referred to Rosita et al. (2020). Once the incubation period was over, the seeds were transferred to pots containing 200 g of zeolite as the planting medium. The plants were maintained for 3 months by watering and fertilizing them with water containing 1.42 g (equivalent to 1420 ppm) NPK (nitrogen, phosphorus, and potassium) fertilizer, following the method outlined in Rosita et al. (2020). There were nine tested treatments , including (1) K1= Pot Culture + *Z. mays*; (2) K2= Pot Culture + *S. bicolour*; (3) K3= Pot Culture + *P. javanica*; (4) F1= Pot Culture + *Z. mays* + 5 spores of *G. fasciculatum*; (5) F2= Pot Culture + *S. bicolour* + 5 spores of *G. fasciculatum*; (6) F3= Pot Culture + *P. javanica* + 5 spores *G. fasciculatum*; (7) E1= Pot Culture + *Z. mays* + 5 spores *C. etunicatum*; (8) E2= Pot Culture + *S. bicolor* + 5 spores *C. etunicatum*; (9) E3= Pot Culture + *P. javanica* + 5 spores *C. etunicatum*. Each treatment was repeated in 25 replicates. After the plants were incubated for 3 months, spore density and AMF colonization rate were observed.

Preparation of NFT hydroponic. The plant was previously inoculated with 5 spores of AMF and incubated for 4 weeks. It was then transferred into a pot containing 200 g of zeolite and placed on the NFT hydroponic equipment (Figure 1). The roots not inoculated by AMF were used as the control (K).

Nutrients were delivered to the plant's roots through a stream of water, allowing the deep plant roots to come in contact with a thin layer of flowing nutrients. The water layer was set up to 3 cm in height, and the plants were maintained for 3 months using 1420 ppm AB Mix Hydro J solution (Tripama & Yahya, 2018) to provide nutrients. Considering of Figure 1. there were 9 treatments tested, including (1) K1= NFT + *Z. mays*; (2) K2= NFT + *S. bicolour*; (3) K3= NFT + *P. javanica*; (4) F1= NFT + *Z. mays* + *G. fasciculatum*; (5) F2= NFT + *S. bicolour* + *G. fasciculatum*; (6) F3= NFT + *P. javanica* + *G. fasciculatum*; (7) E1= NFT + *Z. mays* + *C. etunicatum*; (8) E2= NFT + *S. bicolour* + *C. etunicatum*; (9) E3= NFT + *P. javanica* + *C. etunicatum*. Each treatment was repeated in 25 replicates. The number of spore densities and percent of AMF colonization were measured 3 months after treatment.

Spore density and percent colonization of AMF. In determining the density of AMF spores and their colonization percentage, the wet sieving method was used by using graded filters (425 µm, 212 µm, 106 µm, and 63 µm). The host plant's roots were stained, and then ten root pieces, cut to approximately 1-1.5 cm, were observed under a microscope (Tawaraya et al.,1998). The colonization percentage was calculated based on the presence of external hyphae, internal hyphae, vesicles, arbuscular, and AMF spores on the roots (Rosita et al., 2020).

Preparation of formulated inoculum (F1). The inoculant used in this stage had the highest spore density. The selected inoculant weighed as much as 1 kg and was dried in an oven at 60 °C or 40 °C for 3 hours. It was then mixed with 1.75% Natrium Alginate and 10% *Aloe vera* extract. The concentration of Natrium Alginate and *Aloe vera* is determined by Nurlaeli (2012). The mixture that had changed into granules was dried in the oven at 45 °C for 36 hours.

Preparation of formulated inoculum (F2). The inoculant was dried in an oven at 60 °C or 40 °C for 3 hours and then homogenized by adding 1:1 (v/v) gypsum. Furthermore, clay and water were prepared with a ratio of 1: 2.5 (w/v). Clay and water were mixed until homogeneous and then poured over the surface of the inoculant. Afterward, gypsum was added to the mixture. The mixture of materials was printed with a tool measuring 0.8 cm in diameter and 1 cm in height. The printed tablets were dried in an oven at 45 °C for 36 hours.

Application of formulated inoculum in maize (*Z. mays*). Maize seedlings that had been maintained for 2 weeks were prepared. After that, the formulated inoculum F1 and F2 were applied to the seedlings. There were 9 different types of formulations used as treatments, consisting of: (P1) TZ60F1= AMF + 60 ^{o}C + F1; (P2) TZ60F2= AMF + 60 ^{o}C + F2; (P3) TZ40F1= AMF + 40 ^{o}C + F1; (P4) TZ40F2= AMF + 40 ^{o}C + F2; (P5) TK60F1= 60 ^{o}C + F1; (P6) TK60F2= 60 ^{o}C + F2; (P7) TK40F1= 40 ^{o}C + F1; (P8) TK40F2= 40 ^{o}C + F2; (P9) TK = no treatment. Each treatment was repeated 10 times. The formulated inoculum was applied by making a hole as deep as ± 3 cm. The inoculum was spread over the rhizosphere area of the maize's roots and then covered with sterilized soil. Maize plants were maintained until

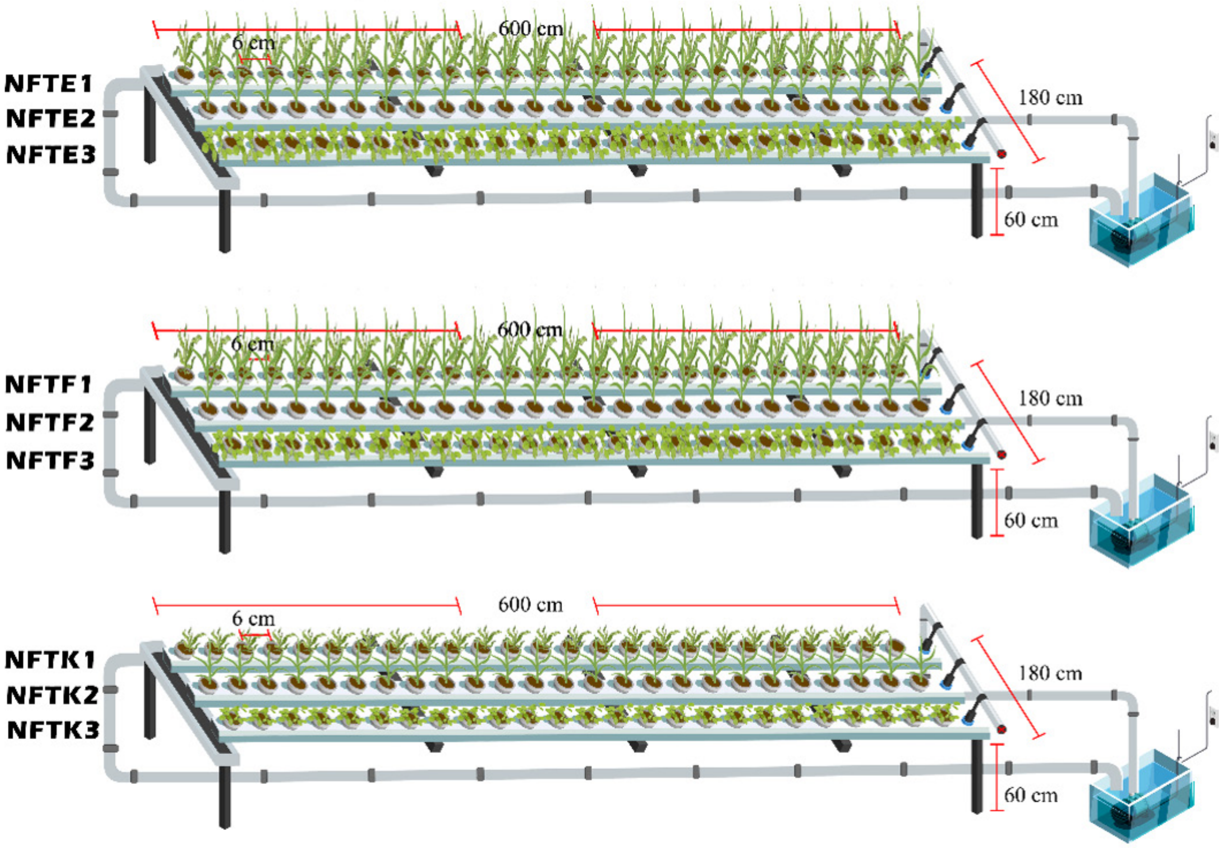

Figure 1. Design of an NFT hydroponic system at the spore production stage

the age of 3 months. Plant height, number of leaves, and dry plant biomass were measured at harvest time. Data were analyzed using SAS software version 9 and further tested using Duncan's Multiple Range Test (DMRT) on a 5% alpha (α) level.

RESULTS AND DISCUSSION

Results of analysis of variance (Table 1) show that the treatments have significant effects on the parameter of spore density (P<0.05). Spore production using the conventional E2 pot culture technique inoculated with *C. etunicatum* achieved the highest spore density value of 350 ± 6.97 spores per 10 g of zeolite. This means that to produce AMF spores successfully, the most suitable technique is needed, especially in selecting the most suitable host plant and its production technique. Therefore, *Z. mays* and Sorghum were chosen as host plants because of their extensive rooting system. Pot culture is an effective method to produce AMF spores, which is carried out by inoculating effective AMF into certain host plants in a sterilized solid medium (Simanungkalit, 2004). Meanwhile, the determination of Pueraria javanica as the host plant was decided according to Aryanto et al. (2018), who reported that interaction among the NFT technique, AB Mix nutrition, and *P. javanica* as the host plant show the best results in spore production and plant biomass.

The pot culture E2 treatment had the highest spore density of 350 ± 6.97 spores per 10 g of zeolite compared to the other treatments and control. However, the percentage of colonization for this treatment was only 50% ± 6.75%. This occurs because the spores are still in the process of germinating within the media, and the hyphae have not yet penetrated into the root tissue. The result is following Rosita et al. (2020), on microscopic observation of *Brachiaria decumbens*, the number of *C. etunicatum* spores reaches 252 ± 9.82 spores per 10 g of zeolite with a percentage of colonization amount of 55% ± 0.06%, whereas AMF colonization does not occur in non-inoculated plants. On the other hand, Rahayu (2014) stated that *C. etunicatum* has an optimal effect on plant growth.

Table 2 shows that treatment affects significantly to AMF spore production (P<0.05). The results revealed that the NFT hydroponic system inoculated by *C. etunicatum* had the highest percentage of AMF colonization at 98% ± 2.66% (E2) compared to other treatments and controls. This indicates that the NFT hydroponic technique significantly enhanced colonization rates. Rodrigues et al. (2021) and Rahayu (2014) reported that *C. etunicatum* effectively colonizes plant roots, achieving a colonization rate of 84%. *C. etunicatum* is effective in colonizing plant roots because of its high ability to infect. *C. etunicatum* and its spores germinated actively to produce hyphae.

Based on Tables 1 and 2, those show that both spore production and colonization data between conventional (using pot culture) and hydroponics (NFT) generally show contradictory results. This shows that AMF has a mechanism to grow and reach nutritional sources. Mycorrhizae respond to and access nutritional sources through hyphal growth mechanisms. Hyphae are long filamentous structures that form mycorrhizal networks. These hyphae grow and branch in extensions in various directions, exploring the growing medium to find and absorb nutrients. The nutrient concentration influences hypha growth in the media. Hyphae tend to grow faster and branch more in directions with a higher concentration of nutrients. This is the reason why the spore density value in treatments using conventional techniques has higher losses than hydroponic techniques because the nutrients are placed below the surface of the media. Meanwhile, hydroponic treatment produces lower spore density values because the nutrients needed for plant growth are dissolved in the air. The percent value of AMF colonization was the highest in treatments given hydroponic techniques. AMF tends to move to the bottom towards sources of water-soluble nutrients.

The spore germination process can be affected by several factors, such as production technique, host plant, and the amount of organic matter in the planting medium. Organic materials can be obtained through plant maintenance activities, including the application of NPK fertilizer (Susanti et al., 2023), which is known to stimulate spore germination. Referring to Akmal's (2019) findings, the root exudate triggers spore germination, particularly the flavonoid compounds of the flavanol type that promote the growth of AMF hyphae. For plants associated with AMF, receiving adequate sunlight to produce high carbohydrate concentrations is crucial. This result is in line with Sieverding (1991), who reported that AMF species, host plants, growing medium, and environmental conditions affect the time of spore formation. Besides that, mycorrhiza produces organic acids which release fixed P (Ristiyanti et al., 2014). One of the factors that influence the development of mycorrhiza is environmental factors. Two environmental factors that influence AMF development are abiotic and biotic. Abiotic factors include climate, light, temperature, soil fertility, and soil pH (Sastrahidayat et al., 2010), while biotic factors can come from the symbiotic host plants with AMF. AMF inoculation increases P uptake and shoots biomass. The contribution of AMF to P uptake and shoot biomass varied based on the phosphate source. The most excellent P uptake was $CaHPO_4.2H_2O$ (Ca2-P). AMF mobilizes phosphates under stress conditions (low P) and increases contact with P in the soil compared with non-mycorrhizal root systems (Yao et al., 2001). The excessive availability of carbohydrates in plant roots decreases soil fertility, so plants cannot absorb nutrients due to limited root systems and an imbalance of one or more macronutrients (N, P, K). The condition formed an association between roots and AMF. Based on the result, when *C. etunicatum* colonized the maize roots, maize plant roots did not enlarge. The success of AMF colonization of plant roots was proven by the formation of external hyphae, internal hyphae, vesicles, and arbuscles (Figure 2).

When hyphae penetrate the cell wall of plant roots, they create distinctive structures, such as vesicles or arbuscles. Vesicles are thin-walled balloons that form at the ends of hyphae and have round or oval shapes. These structures serve as storage organs for nutrient reserves like lipids. Arbuscles, conversely, have tree-like shapes and are formed from intraradical hyphae branches located between the cell wall and membrane. They are crucial in facilitating nutrient and carbon exchange between AMF and host plants. The high and low levels of AMF colonization are influenced by the type of AMF and the shape of plant roots in the form of fibrous roots, taproots, types of roots, and the environmental conditions of these plants (Akmal, 2019).

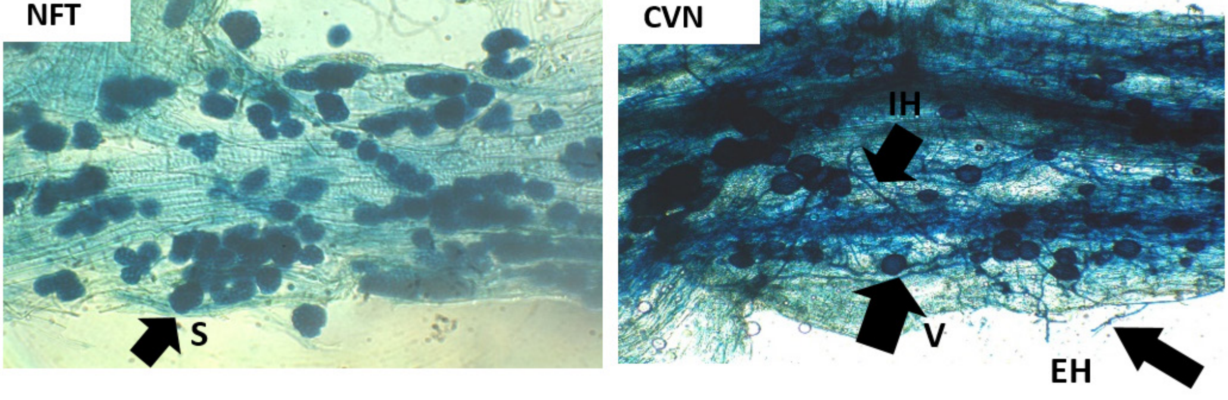

Figure 2. Root tissue microscopic observation of host plant at 3 months after treatment, magnification: 10 x 40 (NFT=hydroponic; CVN= conventional; S= spores; V= vesicles; IH= internal hyphae; EH= external hyphae)

Table 1. Effect of conventional technique in increasing AMF spore production at 3 months after treatment

Code	Treatment	Spore density/10 g of zeolite	Colonization (%)
K1	CVN + *Z. mays*	4 ± 0.58 g	8 ± 0.76 e
K2	CVN + *S. bicolor*	3 ± 0.87 h	8 ± 1.53 e
K3	CVN + *P. javanica*	2 ± 0.96 i	8 ± 1.50 e
F1	CVN + *Z. mays* + *G. fasciculatum*	300 ± 5.16 b	60 ± 4.33 b
F2	CVN + *S. bicolor* + *G. fasciculatum*	190 ± 6.80 d	58 ± 4.65 c
F3	CVN + *P. javanica* + *G. fasciculatum*	198 ± 7.65 c	60 ± 7.94 b
E1	CVN + *Z. mays* + *C. etunicatum*	175 ± 4.38 e	58 ± 5.28 c
E2	CVN + *S. bicolor* + *C. etunicatum*	350 ± 6.97 a	50 ± 6.75 d
E3	CVN + *P. javanica* + *C. etunicatum*	70 ± 4.84 f	70 ± 7.49 a

* The numbers followed by the same letters in the same column are not significantly different based on the DMRT test at the level of α≤5%

Table 2. Effect of hydroponic technique in increasing AMF spore production at 3 months after treatment

Code	Treatment	Spore density/10 g of zeolite	Colonization (%)
K1	NFT + *Z. mays*	2 ± 0.71 g	3 ± 0.91 g
K2	NFT + *S. bicolor*	5 ± 1.15 f	15 ± 1.91 f
K3	NFT + *P. javanica*	5 ± 1.22 f	15 ± 1.29 f
F1	NFT + *Z. mays* + *G. fasciculatum*	10 ± 1.04 e	80 ± 3.58 d
F2	NFT + *S. bicolor* + *G. fasciculatum*	15 ± 1.08 d	80 ± 4.09 d
F3	NFT + *P. javanica* + *G. fasciculatum*	150 ± 4.16 a	60 ± 2.18 e
E1	NFT + *Z. mays* + *C. etunicatum*	20 ± 1.26 c	88 ± 4.52 c
E2	NFT + *S. bicolor* + *C. etunicatum*	50 ± 3.59 b	98 ± 2.66 a
E3	NFT + *P. javanica* + *C. etunicatum*	15 ± 2.45 d	96 ± 3.62 b

* The numbers followed by the same letters in the same column are not significantly different based on the DMRT test at the level of α≤5%

Table 3. Effect of temperature and formulated inoculum application on *Zea mays* vegetative growth at 3 months after treatment

Code	Treatment	Plant height (cm)	Number of leaves	Plant dry biomass (g)
TZ60F1	F1+AMF+60 °C	120 ± 1.70 a	15 ± 0.84 a	26.3 ± 2.46 a
TZ60F2	F2+AMF+60 °C	110.6 ± 6.47 ab	13 ± 1.26 b	20.7 ± 4.37 b
TZ40F1	F1+AMF+40 °C	104.7 ± 8.78 b	12 ± 0.85 c	20.4 ± 5.14 b
TZ40F2	F1+AMF+40 °C	104.4 ± 4.33 b	12 ± 0.70 c	20.06 ± 0.76 b
TK60F1	F1+60 °C	90 ± 4.35 c	9 ± 0.52 d	15.29 ± 3.18 c
TK60F2	F2+60 °C	89.7 ± 2.38 c	9 ± 0.67 d	15.28 ± 1.62 c
TK40F1	F1+40 °C	86.5 ± 3.78 cd	9 ± 0.42 d	15.27 ± 5.29 c
TK40F2	F2+40 °C	80.9 ± 7.82 cd	9 ± 0.42 d	14.53 ± 3.68 c
TK	No treatment	75.4 ± 6.63 d	7 ± 0.42 e	9.03 ± 1.15 d

* The numbers followed by the same letters in the same column are not significantly different based on the DMRT test at the level of α≤5%

The type of root can affect the presence of an AMF. In fibrous roots, more AMF would be found because the roots spread downward and to the side, making it easier for the symbiosis between mycorrhizae and plants (Hermawan, 2015). Host plants from the Graminae class are more suitable for AMF production because they have a higher percentage of colonization and spore density than those from the Leguminosae (Rini & Rozalinda, 2020).

The best AMF inoculant used in the application stage was the conventional technique code E2 (Pot culture + *S. bicolour* + *C. etunicatum*). The highest spore density was 350 ± 6.97 spores per 10 g zeolite. Furthermore, the inoculum was developed by providing treatment in the form of temperature (60 °C, 40 °C) and adding carrier materials (F1: natrium alginate and 10% *Aloe vera* extract; F2: gypsum).

Table 3 shows that inoculum formulations significantly impact the whole of *Z. mays* growth parameters (height, number of leaves, and plant dry weight) of corn plants 3 months after application in sterile soil media. Based on the result, giving formulated AMF inoculum (TZ60F1, TZ60F2, TZ40F1, TZ40F2) can increase the growth of corn plants compared to not giving formulated AMF inoculum (TK60F1, TK60F2, TK40F2, TK). Compared to other treatments and controls, the F1 carrier media (materials in the form of sodium alginate and 10% *Aloe vera* extract), which make up the formulated AMF inoculum treated at a temperature of 60 °C (TZ60F1), had a very significant influence on all growth parameters. The most significant plant height was 120 ± 1.70 cm, the number of leaves was 15 ± 0.84, and the largest plant dry biomass was 26.3 ± 2.46 g (Table 3).

The number of AMF spores and the carrier material play an important role in determining the value of plant growth. The number of AMF spores and the type of carrier material greatly determine the success of inoculation and the effectiveness of the symbiotic relationship between AMF and the host plant. Properly managing these two factors can significantly improve plant growth, health, and productivity. Some carriers contain additional nutrients that can support initial spore growth and increase the effectiveness of inoculation. Adding nutrients in the form of alginate is widely used to control the penetration and stability of adhesives made from starch and latex and to regulate the slow release of chemicals in fertilizers and medicines.

Meanwhile, *Aloe vera* has the potential to add high levels of nutrients to plants, as well as growth stimulants (ZPT) in the form of the hormones auxin and gibberellin, which can increase plant growth. In this research, carrier materials in sodium alginate and *Aloe vera* helped protect *C. etunicatum* spores well; even though they were treated with high temperatures of up to 60°C, AMF could still grow and function well. Previous research reported that *C. etunicatum* has various benefits in increasing growth, nutrient uptake, and plant resistance to abiotic stress (Rosita, 2021). Temperatures above 40 °C are generally too hot for many fungi, including AMF. At very high temperatures, fungal cell proteins and enzymes can denature, disrupting their function and growth. A temperature of 60 °C will damage cellular structures and kill AMF and many other soil microorganisms. AMF can grow well at 30ºC, and the best development of spores to produce mycelia occurs at 28ºC. Temperature can affect the growth and development of AMF. High temperatures affect the growth and formation of mycorrhizal colonies. The development of most mycorrhizal fungi is inhibited if the soil temperature is below 5ºC and the temperature above the soil surface is 35ºC. If the soil temperature reaches 50ºC, it can kill mycorrhizal fungi. From this statement, data was obtained that if the soil temperature reaches 50ºC, it can cause death of AMF.

A good temperature for AMF development is 30ºC, but for mycelial colonization, the best temperature is 28 - 34ºC (Rumiatun, 2024). Inoculum based on G. intraradices spores could remain infectious in moist soil for up to three weeks at temperatures as high as 380C (Haugen & Smith, 1992). The percentage of colonization usually rises in experimental systems between 100 and 300C. However, some plant-fungus combinations can thrive at much lower or higher temperatures (Bowen, 1987). Aryanto et al. (2018) stated that 30°C is the optimal temperature for AMF growth, which can increase spore production in plant feed such as grass. The optimal soil temperature for AMF spore production is usually above the optimal temperature of the host plant. Temperatures below 15°C can inhibit mycorrhizal colonization, while mycorrhizal activity increases with increasing soil temperature. Temperature and nutrition influence the growth and production of spores, which greatly affect the quality and quantity of spore production. In the hydroponic technique, an irrigation system that uses nutrient solutions with the right concentration can increase the production of AMF biomass and spores. The interaction between temperature and nutrition is very significant in AMF spore production. Optimal temperatures and adequate available nutrients can increase spore production. In hydroponic techniques, of using an appropriate irrigation system and balanced nutrition can maximize AMF spore production (Aryanto et al., 2018).

CONCLUSION

The conventional pot culture technique effectively increases AMF spore density, and the NFT hydroponic technique effectively increases root colonization percentage. The E2 treatment with *C. etunicatum* in pot culture generated the highest spore density of 350 ± 6.97 spores per 10 g of zeolite ($P<0.05$) compared to other treatments and control. Meanwhile, the hydroponic technique had the highest AMF percentage colonization with the E2 treatment at 98% ± 2.66% ($P<0.05$). AMF has a mechanism to grow and reach nutritional sources. Spore density value in treatments using conventional techniques has higher losses than hydroponic techniques because the nutrients are placed below the surface of the media.

The formulated AMF inoculum (TZ60F1, TZ60F2, TZ40F1, TZ40F2) can increase the growth of *Zea mays* compared to not giving formulated AMF inoculum (TK60F1, TK60F2, TK40F2, TK). Compared to other treatments and controls, the F1 carrier media (sodium alginate and 10% *Aloe vera* extract), which makes up the formulated AMF inoculum treated at a temperature of 60 °C (TZ60F1), had a very significant influence on all growth parameters. The most significant plant height was 120 ± 1.70 cm, the number of leaves was 15 ± 0.84, and the largest plant dry biomass was 26.3 ± 2.46 g. Carrier materials in sodium alginate and *Aloe vera* helped protect *C. etunicatum* spores well; even though they were treated with high temperatures of up to 60°C, AMF could still grow and function well.

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