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RESEARCH

The leaf numbers and root diameter of *Pule Pandak* (*Raufolvia serpentina Benth*.) influence the root-dry weight and reserpine content

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Abstract
Reserpine is the most important alkaloid content in *Rauvolfia serpentina Benth's.* (*Pule Pandak*) root. To figure out the relationship of *Pule Pandak* morphological character with the reserpine contents, an observation based analysis was done. The environment, fertilizing treatment, and fenotipe observation data were collected and analyzed. In *Pule Pandak* cultivated at 600 m – 450 m above sea level under the teaks (*Tectona grandis*) or sengon (*Paraserianthes falcattaria*), in latosol soil and fertilizer doses at 20 t/ha – 30 t/ha, we found that the leaf numbers and root diameter would followed by the root's dry weight and the increase of root diameter will be followed by the increase of its reserpine content.

Key words: *Raufolvia serpentine*, *Pule Pandak*, Reserpine, Root

INTRODUCTION

Raufolvia serpentina Benth. has many names. Its original name in India region is "Pagal–ki–dawa" aka "Chandrinkan" aka "Chotachand", and its name in Sanskrit called as "Sarphagandha", in China known as "yin tu luo fu mu" and in England as "Snake Root". In Java Indonesia the *R. serpentina Benth*. plant is known as "Pule Pandak" and in Sunda as "rat root" since it has a straight rooting, rare branches and smaller towards its end like a rat's tail.

Raufolvia plants are distributed among tropical areas such as Central and South America, Africa, India, Thailand. Myanmar, Malaysia, Sumatera and Java, but the biggest numbers are in Africa and South America. *Raufolvia* is also reported to be found in China and Japan. From those above mentioned locations, *Raufolvia serpentina* is only found in India, Indonesia (Java), Myanmar, Thailand and Malaysia. *Pule Pandak* itself is an essential plant which becomes a concern after the discovery of its alkaloid. The use of *Pule Pandak* as a traditional medicine for curing diseases has been applied since hundred years ago [(1)].

The value of *Pule Pandak* as medicinal plant is on its alkaloid content. *Pule Pandak* part that contains a lot alkaloid is the root. The root contains more than 60 types of alkaloid like reserpine, yohimbine, serpentine, and ajmalin. In point of the alkaloids content, the reserpine is the most important since it used as anti-hypertensive drug. Recently three more new alkaloids had been successfully isolated, included in indol monoterpenoid alkaloid of this plant. The alkaloid content is mainly depending on the environment factors (e.g., type of soil, nutrient, rainfall, temperature and light) that affect the enzymatic process. To get

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the maximum photosynthesis it is important to have a high dry weight environment.

In its nature *Pule Pandak* lives in 1,000 m above sea level lowland, in C type of climate according to the Schmidt-Ferguson, living on regosol, mediteranian and litosol soil. It is sheltered endemic under the teaks. According to Sarin [2], the plant is not resistant to heavy rain; therefore its growth is limited since the heavy rainfall makes all of the leaves fall so need time to grow it back. Using paranet as shading it could gave the highest dry weight gained with also high reserpine (50 % - 80 %) [3, 4]. Giving an organic fertilizer 20 t/ha would increase the root's dry weight and reserpine volume gained [1]. Ex-situ maintenance by *Pule Pandak* plant cultivation needs suitable locations for its growth and development to gain high plant productivity. The use of shelter under the standing trees becomes an option in agroforestry plant pattern.

Classification system based on morphology is commonly used since it is easy and less time consumed. However, it has weakness since the methods could not neglect the influence of environment factor, plant age and tissue difference.

MATERIALS AND METHODS

The research was done in Tekil Village Wonogiri Regency (for took the sample of Pule Pandak and grew habitation), Butuh Village Sawangan District, and KPH Saradan Madiun Regency (for cultivated the plants). Environment observation at Tekil Village were the height of the place, climate factor towards rain volume, light intensity, air temperature, air humidity, soil plasticity and also did a soil analysis.

We also performed a fertilizing treatment using an organic fertilizer; 10 t/ha, 20 t/ha, 30 t/ha with planting distance 30 cm X 30 cm. A variant analysis and F test were performed with Duncan test in 5 % value. Afterward, a correlation and regression analysis was done to figure out the relation between the above measurement point toward to the root yield and its reserpine content.

RESULTS AND DISCUSSION

In the time observation, we found that the *Pule Pandak* in Tekil did not always live together with the teaks, but sometimes it grew among other low bushes such as *Tembelekan* (*Lantana camara L.*), *Alang-alang* (*Imperata cylindrica Beauv.*), *Kaliandra* (*Caliandra sp.*) and other trees than teaks (Table 1).

Table 1. The variety of Pule Pandak in Tekil forest habitat

Parameter	Average
Height (cm)	30.1
Number of leaf	19
Width of leaf (cm^2)	382.58
Total root length (cm)	47.92
Biggest root diameter (mm)	8.3
Fresh Root weight (g)	35.94
Dry Root weight (g)	23.51
Amount of Reserpina (ppm)	174.6

We found a positive correlation between the leaf numbers with the root diameter (r = 0.52*) and dry weight (r = 0.50*) which means the increase of the leaf numbers was followed by the increase of the root diameter and dry weight (Table 2). The number of the root had a positive correlation to the dry weight and reserpine contents. However the diameter of the root had a negative correlation to length of the root. Abbas and Hay [6] stated that the increase of the root number would decrease the root diameter and length. The increase of root diameter would be followed by the increase of root dry weight and reserpine volume.

Sawangan location was higher than Tekil and Saradan. This different place height affected the air temperature and humidity under the standing trees. Light intensity difference was mostly influenced by the types of standing trees; in Tekil and Saradan were located under the Teaks while in Sawangan was under standing trees of Sengon so that the leaf shape of the standing trees affected the light intensity which extended to below the standing trees (Table 3).

The increase of air humidity would increase the soil water and decrease the soil temperature. Monteith et al. [7] stated that the shading increases the efficiency of water used by the plants below, by decreasing the evapo-transpiration of the plants below.

The soil chemical character of those three research location were not so much different and it could be categorized into less fertile soil (Table 4). Soil in Tekil, Sawangan and Saradan were latosol. NPK content and organic substances in those three research field showed the low availability of the elements. However, KTK in Saradan was higher than that of in Tekil and Sawangan.

Table 2. Correlation coefficient of *Pule Pandak* from Tekil forest habitat

	TT1	JMD1	LSD1	PA1	JA1	DMT1	BKA1
JMD1	-0.38						
LSD1	-0.64**	0.73**					
PA1	0.35	0.27	-0.30				
JA1	-0.04	-0.04	-0.30	0.55*			
DMT1	0.11	0.52*	0.35	-0.58*	-0.50*		
BKA1	0.35	0.50*	0.06	0.84**	0.08	0.64**	
Rsp1	0.07	0.05	0.32	0.19	0.34	0.78**	0.272

Note: TT = height of plant; JMD = number of leaf; LSD = width of leaf; PA = root's length; JA = number of root; DA = root's diameter; BKA = root's dry weight; RSP = Reserpine

Table 3. The environment variable on research location

Location	Place Height (m asl)	Rainfall (m/yrs)	Climate Type	Light Intensity (fc)	Air Temperature (˚C)	Air Humidity (%)	Soil Water (%)
Tekil	400	1.966.40	C	11.873	27.46	77.67	36.48
Sawangan	790	2980.50	C	10.928	24.37	88.44	39.18
Saradan	435	1854.45	C	12.870	27.64	78.24	38.48

Table 4. Analysis of soil sample on research location

Soil Character	Tekil	Sawangan	Saradan
Chemical Characters			
Total N (%)	0.31	0.48	0.43
Provided P (ppm)	5.53	16.23	11.76
Exchanged K (me/100g)	0.18	0.19	0.24
Ca (me/100g)	19.25	10.42	19.48
KTK (me/100 g)	18.60	19.37	30.15
Organic matter (%)	3.05	4.02	3.39
PH	5.92	5.46	5.93
Textures:			
dust (%)	39.20	32.43	33.07
clay (%)	42.36	52.34	52.76
silk (%)	18.44	15.23	14.17
Water capacity	44.96	43.77	42.37
	Latosol	Latosol	Latosol

Generally, the dose of the organic fertilizer had an effect on the entire growth and yield parameter in Sawangan and Saradan but of the plant height, root length and reserpina in Sawangan (Table 5).The increase of the root result was not always enclosed by the increase of secondary metabolite (reserpine) (Figure 1).

Herms and Mattson [8] stated that there is a dilemma for the plant growth and the alkaloid forming. To stimulate the forming of defense function like the secondary metabolite it would needs an environment pressure condition. However, enough fertilization and soil water is able to increase the alkaloid production [9], therefore the fertilization could affects the reserpine amount as proved by the present data. We found a direct relationship between the leaf number and the root dry weight (r = 0.49*). The increase of the leaf number was followed by the increase of the root dry weight (Table 6).

Tabel 5. Analysis of variance of growth and yield parameter of *Pule Pandak*

		KT							
Location		TT	JMD	LSD	PA	JA	DA	BKA	RSP
Sawangan	P	ns	**	**	ns	*	*	*	ns
Saradan	P	**	*	*	*	*	*	*	*

Note: P= Fertilization; TT = height of plant; JMD = number of leaf; LSD = width of leaf; PA = length of root; JA = number of root; DA = diameter of root; BKA = dry weight of root; RSP = reserpine

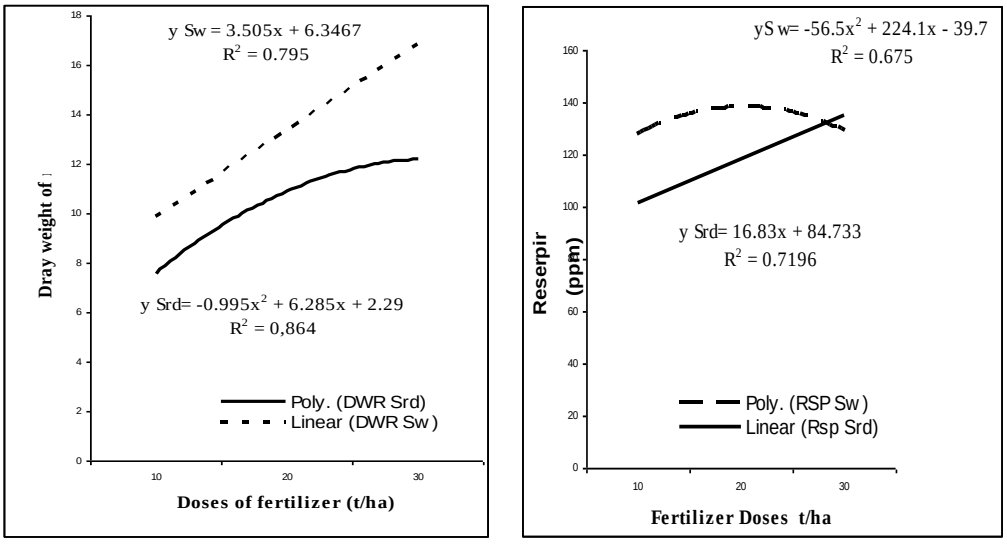

Figure 1. Correlation of the fertilizer doses with the dry weight of the root and the reserpina content of *Pule Pandak*

Table 6. The cross – component correlation of growth and root with dry weight of root (Sawangan)

Parameter	JMD	LSD	PA	JA	DA	r (BKA)
JMD	**0.49***	-0.14	0.31	-0.02	0.22	0.46*
LSD	0.24	**0.40***	0.08	-0.06	0.20	0.86**
PA	0.10	-0.16	**-0.18** tn	0.07	0.05	-0.12 tn
JA	-0.10	-0.18	0.07	**0.48***	0.17	0.57*
DA	0.19	-0.29	0.18	-0.17	**0.59***	0.50*

Note: r(x_iy) BKA: Correlation coefficients towards root dry weight. The bold shows direct correlation, without bold shows undirect correlation; tn=not significant; **/* = significant on 1 % and 5 %, JMD = number of leaf; LSD = width of leaf; PA = root length; JA = number of root; DA = root diameter; BKA = root dry weight.

The leaf number influent the root diameter and the increase of root number was not always followed by the increase of root dry weight. The increase of the root diameter was followed by the increase of the reserpine content (Table 7) and the increase of the root diameter was followed by the increase of the root dry weight (Table 8).

Finally, the increase of the root diameter increase was followed by the increase of the reserpine volume (Table 9).

Table 7. The cross – component correlation of growth and root with reserpine amount (Sawangan)

Parameter	JMD	LSD	PA	JA	DA	r (Rsp)
JMD	**0.30tn**	0.21	-0.10	-0.11	0.11	0.41tn
LSD	0.28	**0.46***	-0.09	-0.18	0.19	0.66*
PA	-0.18	-0.12	**-0.39***	-0.09	0.09	-0.51*
JA	0.11	0.08	-0.15	**-0.21tn**	0.08	-0.09tn
DA	0.22	0.12	- 0.28	-0.11	**0.59***	0.54*

Table 8. The cross – component correlation of growth and root with dry weight of root (Saradan)

Parameter	JMD	LSD	PA	JA	DA	r (BKA)
JMD	**0.41***	-0.19	0.17	-0.11	0.28	0.56*
LSD	0.10	**0.24tn**	-0.11	-0.20	0.11	0.14tn
PA	-0.31	0.07	**-0.29tn**	-0.16	0.02	-0.67**
JA	0.30	-0.14	-0.13	**0.39***	0.15	0.57*
DA	0.27	-0.16	- 0.09	0.12	**0.57***	0.55*

Table 9. The cross – component correlation of growth and root with reserpine amount (Saradan)

Parameter	JMD	LSD	PA	JA	DA	r (Rsp)
JMD	**0.44***	0.12	-0.06	0.09	0.28	0.67**
LSD	0.14	**-0.19tn**	-0.18	0.17	0.21	0.15tn
PA	-0.21	-0.19	**-0.39***	0.04	0.22	-0.53*
JA	0.24	-0.18	-0.16	**0.41t***	0.29	0.60**
	DA	0.08	- 0.29	0.12	**0.51***	0.61**

From the location test of the *Pule Pandak* plant cultivation on 450 m - 600 m above of the sea level, under the Teaks (*Tectona grandis*) or Sengon (*Paraserianthes falcattaria*), in latosol soil and fertilizer doses at 20 t/ha – 30 t/ha, it can be concluded that the increase of the leaf number and the size of root diameter was followed by the increase of root's dry weigh, and the increase of the root's diameter size was followed by the reserpine content. Therefore it would be necessary to do a molecular research in the future to role out the relationship between the molecular and morphological characters of the *Pule Pandak*.

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