Agricultural waste materials as component of organic potting media for the endangered *Nepenthes truncata* Macf. (Philippine pitcher plant)

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Abstract: Agricultural waste materials, namely, coconut fiber (CF), coir dust (CD), and charcoaled rice husk (CRH) taken singly or in combination with commonly used organic substrates wood charcoal chips (WCC), chopped tree fern (CTF), sphagnum moss (SM), and osmunda fiber (OF) were characterized for their suitability as components of mixed potting media for *Nepenthes truncata* (Macf.) plantlets. Several mixed media were evaluated, each with a component for aeration (WCC, CTF, or CRH), moisture retention (CF, CD or SM), and drainage (OF). Conventionally used container media mixes served as controls. Based on survival and leaf condition of plantlets, CD could replace SM, and both were superior to CF in moisture retention, while CRH was superior to wood charcoal chips and tree fern for aeration. Both CD and CRH are locally available and inexpensive compared to the materials they could replace. Small-sized plantlets performed better in Treatment 6 (T6) (2:1:1 mixture of SM, OF, and CRH) and Control 1 (pure SM) while medium-sized plantlets only preferred T6. Physico-chemical properties of T6 suggest that the appropriate medium for *N. truncata* plantlets is slightly acidic with low electrical conductivity, high porosity, moderate water holding capacity, and moderate water retention efficiency.

Introduction

Nepenthes truncata is an endemic plant of the Philippines, particularly in Mindanao (McPherson & Amoroso 2011). It is now considered as a commercially valuable ornamental but is classified as endangered by the IUCN (Clarke *et al*. 2006) due to slash and burn farming methods, over-collection from the wild, and poor cultivation practices of other crops such as vegetables and ornamentals. A suitable artificial media for cultivation of *N. truncata* will contribute to a technology package for propagated plantlets and reduce plant collection from the wild. All *Nepenthes* species naturally grow in low-nutrient habitats such as peat swamp, mountain rainforests, and pure sand (Horman 2000). In nurseries, soilless mixes composed purely of organic substances are commonly used as growing media (Greer 1998). In previous studies on cultivation of *Nepenthes* species, the growing media recommended were long fiber sphagnum moss (Butler 1990), a combination of osmunda fiber and sphagnum moss (De Kanel & Smith 1992), and perlite in combination with long fiber sphagnum moss and peat moss (Rice 2006).

Waste products are now being utilized as components in plant growing media. Among these, pruning waste compost (PWC) (Hernández-Apaolaza *et al*. 2005) and 7:3 peat-rice husk mixture (Marianthi 2006) have been used for pines, PWC plus spent mushroom compost (SMC) for ornamental plants (Benito *et al*. 2005), and compost from biosolids combined with yard trimmings for *Pachystachys lutea* Nees (Wilson & Stofella 2000). Coconut fiber and coir dust have been used as substitutes for peat in many studies (Abad *et al*. 2002; Noguera *et al*. 2000; Wilson & Stofella 2000) due to properties similar to peat (Lennartson 1997). However, the suitability of coconut fiber or coir dust as growing media for epiphytic *Nepenthes* species has not yet been explored. Reuse of waste materials as alternative to other organic container substrates can help lessen the need for extracting resources from natural habitats, lessen residue accumulation in the environment (Raviv *et al*. 1986) and cut down the cost of growing media (Ingelmo *et al*. 1998). The study was conducted to test the suitability of agricultural waste materials (CF, CD, and CRH) in combination with locally available organic substrates (SM, OF, CTF, or WCC) for growing *N. truncata* in the nursery.

Materials and Methods

Physico-chemical characterization of media and their components. The mixed media as well as their components were characterized with three replicates per test. Determination of the water-holding capacity was done through a modification (use of hanging pot instead of a clay pot) of the procedure described by Holcomb (2004). For porosity (total and aeration) characteristics, a modification (use of hanging basket lined with plastic sheet instead of clay pot) of the procedure in the Nursery Production Factsheet by MAFF (2001) was followed. Water-retention efficiency of the potting mixes was determined by adopting the procedure of Nursery and Garden Industry Australia (2002). Electrical Conductivity (EC) and pH characterization was done by the Department of Agriculture, Davao City using 1:2 extraction method (Argo 2004).

Plant Material. Plantlets of *N. truncata*, collected from the wild and maintained for 3 months in a uniform medium of sphagnum moss at the Fuertes Nursery in Marilog District, Davao City, were used in the experiments (Fig. 1). Two arbitrary size categories were evaluated in the experi-

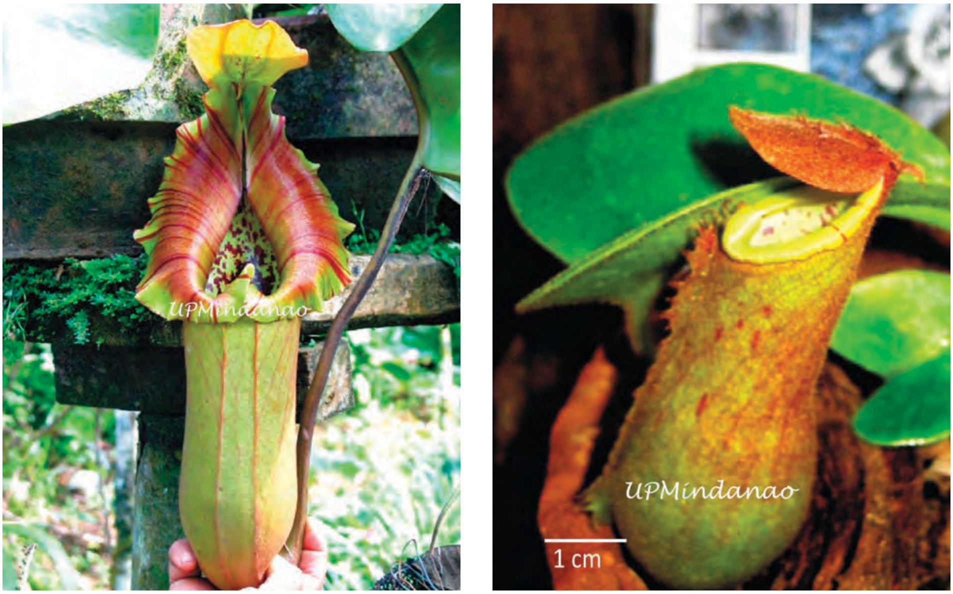

Figure 1: *Nepenthes truncata* mother plant (left) and representative plantlet used in the experiment (right).

Table 1. Composition of experimental organic potting media treatments for *Nepenthes truncata* cultivation.				
Treatment	Components (% by volume)			
	Aeration (25)	Moisture retention (50)		Drainage (25)
	Experiments 1 & 2	Experiment 1	Experiment 2	Experiments 1 & 2
1	WCC	CF	CD	OF
2	CTF	CF	CD	OF
3	CRH	CF	CD	OF
4	WCC	SM	SM	OF
5	CTF	SM	SM	OF
6	CRH	SM	SM	OF

CF-coconut fiber; OF- osmunda fiber; WCC- wood charcoal chips; CTF- chopped tree fern root; CRH-charcoaled rice husk; SM- sphagnum moss

Table 2. Percentage of survival[1] of *Nepenthes truncata* Macf. plantlets in different potting mixes from January to July 2005.							
Code	Potting mix	Mean % Survival of plantlets at specified interval (days)[2]					
		30	60	90[3]	120	150	180
C1	Control 1	100	100	100a	100	100	100
C2	Control 2	100	90	90ab	90	90	90
C3	Control 3	100	100	90ab	80	70	50
C4	Control 4	90	80	70ab	60	50	40
T1	CF-OF-WCC	100	70	60b	30	10	10
T2	CF-OF-CTF	100	80	80ab	30	30	20
T3	CF-OF-CRH	100	100	100a	80	80	70
T4	SM-OF-WCC	100	100	90ab	80	80	80
T5	SM-OF-CTF	100	90	70ab	60	60	60
T6	SM-OF-CRH	100	100	100a	100	100	100
GRAND MEAN		99	91	85	71	67	62
P value (potting mix × plant size)		0.447	0.070	0.016	0.090	0.216	0.113

[1] data were transformed using square-root transformation
[2] means within columns without letters are not significantly different at alpha=0.05
[3] means with common letter(s) are not significantly different using DMRT at alpha=0.05.
Control 1: 100% long fiber sphagnum moss;
Control 2: 50% peat moss, 25% perlite, and 25% pine tree bark;
Control 3: 33.33% river pebbles, 33.33% tree fern root, and 33.33% wood charcoal;
Control 4: 66.67% sphagnum moss, and 33.33% tree fern root
CF: 50% coconut fiber; OF-25% osmunda fiber; WCC- 25% wood charcoal chips; CTF- 25% chopped tree fern root; CRH-25% charcoaled rice husk; SM- 50% sphagnum moss

Table 3. Comparison of percentage of survival[1] of small and medium *Nepenthes truncata* Macf. plantlets in different potting mixes from January to July 2005.

Plantlet size	Potting mix	Code	% Survival of plantlets at specified interval (days)[2]					
			30	60	90[3]	120[3]	150[3]	180[3]
Small	Control 1	C1	100	100	100a	100a	100a	100a
	Control 2	C2	100	80	80ab	80ab	80ab	80a
	Control 3	C3	100	100	100a	80ab	80ab	60ab
	Control 4	C4	80	60	40bc	40bc	40bc	20bc
	CF-OF-WCC	T1	100	40	20c	0c	0c	0c
	CF-OF-CTF	T2	100	60	60abc	0c	0c	0c
	CF-OF-CRH	T3	100	100	100a	100a	100a	100a
	SM-OF-WCC	T4	100	100	80ab	60ab	60ab	60ab
	SM-OF-CTF	T5	100	80	60abc	60ab	60ab	60ab
	SM-OF-CRH	T6	100	100	100a	100a	100a	100a
Mean			98	82	74	62	62	58
P value (across potting mixes)			0.456	0.086	0.015	0.000	0.000	0.000
Medium	Control 1	C1	100	100	100	100	100a	100a
	Control 2	C2	100	100	100	100	100a	100a
	Control 3	C3	100	100	80	80	60ab	40ab
	Control 4	C4	100	100	100	80	60ab	60ab
	CF-OF-WCC	T1	100	100	100	60	20b	20b
	CF-OF-CTF	T2	100	100	100	60	60ab	40ab
	CF-OF-CRH	T3	100	100	100	60	60ab	40ab
	SM-OF-WCC	T4	100	100	100	100	100a	100a
	SM-OF-CTF	T5	100	100	80	60	60ab	60ab
	SM-OF-CRH	T6	100	100	100	100	100a	100a
Mean			100	100	96	80	72	66
P value (across potting mixes)			No Value (subsets cannot be computed with alpha=0.05)		0.543	377	0.048	0.010

[1] data were transformed using square-root transformation
[2] means within columns without letters are not significantly different at alpha=0.05
[3] means within columns with common letter(s) are not significantly different using DMRT at alpha=0.05.
Control 1: 100% long fiber sphagnum moss;
Control 2: 50% peat moss, 25% perlite, and 25% pine tree bark;
Control 3: 33.33% river pebbles, 33.33% tree fern root, and 33.33% wood charcoal;
Control 4: 66.67% sphagnum moss, and 33.33% tree fern root
CF: 50% coconut fiber; OF-25% osmunda fiber; WCC- 25% wood charcoal chips; CTF- 25% chopped tree fern root; CRH-25% charcoaled rice husk; SM- 50% sphagnum moss

ments: small and medium. Small plantlets have internode length (IL) ≤1 cm with at least 6 nodes while medium plantlets have either IL <1 cm with at least 9 nodes or with IL >1 cm with at least 4 nodes.

Experimental conditions for evaluation of nursery media. The experiments were conducted at a nursery in Marilog District, Davao City with a site elevation of 1200 m and a temperature range of 18 to 27°C. Light intensity ranged from 2200 to 18480 lux (SPER SCIENTIFIC 840020 digital light meter). Experiment 1 was conducted during the dry season (January to July 2005) with relative humidity range of 50.2 to 84.6% (VMR Traceable® Hygrometer), and average monthly rainfall of 101.57 mm as measured by the Philippine Atmospheric, Geophysical and Astronomical Services Administration (PAGASA) in Lanang, Davao City. Experiment 2 was conducted during the wet season (August to December 2005) with relative humidity range of 60.8 to 85.6% and average monthly rainfall of 155.52 mm.

Fertilizer application and watering. All plantlets were sprayed monthly with commercial foliage orchid fertilizer (Manusol®), with an NPK ratio of 19:19:19 and Mg, Cu, Mn, Zn micronutrients. Each formulation contained two tablespoons (about 30 g) of the fertilizer dissolved in 48 L of rainwater to obtain a concentration that was only one-third of the ordinary dosage for orchids to prevent over fertilization of the plantlets. Watering was done daily except during rainy days. Either spring water or stored rainwater was used and application was done using a hand-held atomizer.

Pest and fungi control. All plantlets were sprayed monthly with insecticide and fungicide using commercial organophosphate insecticide, profenofos (Selecron®), a systemic fungicide, metalaxyl-m (Ridomil®), and a broad-spectrum fungicide, mancozeb (Dithane®). Individual solutions of the chemicals were initially prepared prior to preparation of the spray solution as follows: Insecticide solution was prepared by mixing 1 ml profenofos (0.5 $g \cdot L^{-1}$ active ingredient) to 1 L rainwater; systemic fungicide solution consisted of 3.9 g metalaxyl-m (0.0098 $g \cdot L^{-1}$ active ingredient) added to 1 L rainwater; and broad-spectrum fungicide solution consisted of 1 g mancozeb (1.6 $g \cdot L^{-1}$ active ingredient) to 1 L of rainwater. All solutions were thoroughly mixed and transferred to individual plastic bottles. To prepare the spray solution, 500 ml of each chemical solution was mixed. A spreader sticker (Hoestick®) was added to the pesticide and fungicide mixture at a concentration of 0.53 ml L-1, to allow even distribution of the chemicals on the plant's surface. The insecticide-fungicide-sticker mixture was applied to the plantlets using a plastic hand-held atomizer.

Experiment 1: Use of coconut fiber as organic medium component. There were separate setups for small and medium plantlets, each laid out in a Completely Randomized Design (CRD) with four controls (commercial potting mixes), six experimental treatments, and five replicates, with one plant per replicate. The control treatments were: (C1) long fiber SM (Catalani 2000); (C2) 2:1:1 mixture of peat moss, perlite, and pine tree bark (Sarracenia Northwest 2012; Bilderback 2002); (C3) 1:1:1 mixture of river pebbles, tree fern root, and WCC (Vogelpoel 1980; Malesiana Tropicals n.d.); and (C4) 2:1 mixture of SM and CTF tree fern root (Malesiana Tropicals n.d.). The compositions of the experimental potting media are shown in Table 1. Survival and condition of the plantlets were monitored monthly for five months.

Experiment 2: Use of coconut coir dust as organic medium component. Due to high mortality rates observed in potting mixes with CF in Experiment 1, CF was replaced with CD (Table 2), which has physico-chemical properties comparable to SM (Cresswell 2001), in Experiment 2. The same four controls used in Experiment 1 were also used in Experiment 2, thus the total number of treatments in a single-factor analysis was 10. To determine the contributions of various components to the plant response, the 6 experimental media were also analyzed as 2 × 2 × 3 factorial experiment, where the factors were plant size (small or medium), component for moisture-retention (CD or SM), and component for aeration (WCC, CTF, or CRH), respectively. As in Experiment 1, the component for drainage (OF) was held constant. Survival and condition of the plantlets were monitored monthly for five months. The condition of the leaves and pitchers were evaluated using a 1 to 5 rating scale where 1 is least and 5 is best.

Data Analyses. Statistical analyses were done using Analysis of Variance (ANOVA) of a CRD and mean separation was done using DMRT. Pearson's Correlation Analysis was used to test for correlation between physical properties of potting mixes. For the percentage survival, data was transformed using square-root transformation.

Results and Discussion

Experiment 1: Use of coconut fiber as organic medium component.

The experiment suffered from moisture stress due to lack of rain and ineffective manual watering. At 60 days, there was a marked reduction in survival in some treatments; and in 90 days, there were statistically significant differences. C1 and T6 gave the highest and most consistent survival rates while T1 gave the lowest (Table 2).

The survival rates of small plantlets were lower than that of medium plantlets (Table 3). Severe symptoms of water stress such as yellowing of leaves and curling of leaf blades accompanied low survival rates. No new shoots were produced and lower leaves tend to undergo abscission.

Plantlets grown in coconut fiber-based mixes have respectively lower survival rates of 46.7% ($p=0.007$), 40% ($p=0.001$), and 33.3% ($p=0.00$) for 120, 150, and 180 days after transfer compared to a consistent 80% survival rate for plantlets grown in media with sphagnum moss.

Experiment 2: Use of coconut coir dust as organic medium component

A 100% survival after 150 days of observation was obtained in all potting mixes in contrast to the varied survival rates of the plantlets in Experiment 1. This is not only due to the use of CD instead of CF in the growing medium but also due to higher amount of rainfall when Experiment 2 was conducted. There was no significant difference among the controls (C1 to C4) in terms of leaf condition. However, among 6 experimental treatments, T1 with CD was inferior ($p=0.01$) compared to potting mixes T3 with CD, T5, and T6. Treatments T5 and T6 were also significantly better in leaf condition ($p=0.01$) than all 4 controls and the other experimental treatments. Of the three factors (plant size, component for moisture-retention, and component for retention) only plantlet size had a significant effect on the condition of *N. truncata* leaves. Throughout the experiment, the leaf condition of the small plantlets were significantly better than that of medium plantlets ($p=0.03$). Coir dust and sphagnum moss were comparable, while rice husk was better than wood charcoal and tree fern among the components for aeration.

Physico-chemical characterization of organic substrates and potting mixes in relation to plant growth. A growing medium with EC readings below the critical level of 2.0 mmhos·cm^{-1} is considered a good substrate for the plant (A & L Plains Agricultural Lab, Inc. 2001; Argo 2004; Harris Laboratories 2009). Other suitable physico-chemical properties include: pH between 6.5 and 7.0 but some species have developed adaptations that allow them to thrive at a higher (Mauseth 1998) or lower pH levels like *Nepenthes* (Polunin 1994), WHC within 0.60 to 0.80 g·ml^{-1} (Strain 1999), WRE values greater than 80% (NGIA 2002), AP of at least 20 to 25% and as high as 45% in warmer temperatures with TP that must be greater than 60 to 70% (MAFF 2001). Based on physico-chemical analyses, the EC indicate that all the potting substrates and potting mixes are suitable. Since *N. truncata* plantlets prefer a slightly acidic medium (Polunin 1994), the majority of the mixes are found suitable except T1 with CF, T4, and C3, which are basic. WHC and WRE were highly correlated and

Table 4. Physico-chemical properties of different potting media for plantlets of *Nepenthes truncata* Macf.

Potting Mixes	Code	Physico-Chemical Properties[1]					
		AP	TP	WHC	WRE	EC	pH
Control Treatments							
Control 1	C1	61.02a	75.23a	0.26f	25.70bcd	0.72	6.6
Control 2	C2	38.09a	59.17a	0.45c	16.70e	0.33	5.1
Control 3	C3	42.13a	60.39a	0.02h	20.00de	0.24	7.8
Control 4	C4	52.59a	63.94a	0.32d	1.33f	0.63	6.6
Experimental Treatments							
CF-OF-WCC	T1 with CF	52.79a	69.68a	0.06e	2.33e	0.51	8.4
CF-OF-CTF	T2 with CF	49.53a	63.46a	0.03e	4.33e	0.27	6.0
CF-OF-CRH	T3 with CF	43.26a	61.16a	0.17d	11.00d	1.15	5.3
CD-OF-WCC	T1 with CD	40.23bc	53.67b	0.46c	30.00ab	0.44	6.8
CD-OF-CTF	T2 with CD	42.23abc	48.92b	0.67a	28.30abc	0.32	6.6
CD-OF-CRH	T3 with CD	41.14bc	42.96b	0.63bc	32.30a	0.31	6.2
SM-OF-WCC	T4	60.03a	80.62a	0.21g	27.30abc	0.60	7.8
SM-OF-CTF	T5	52.16a	63.26a	0.28ef	16.00e	1.02	6.7
SM-OF-CRH	T6	61.58a	77.72a	0.29de	23.30cd	0.85	6.0
Mean		48.98	67.46	0.36	22.10	0.57	6.6
P value		0.03	0.03	0.00	0.00		
Coefficient of Correlation[2]		0.00[3]		0.00[4]			

[1] means within columns with common letter(s) or no letters are not significantly different using DMRT at alpha=0.05.

[2] correlated at 0.05 level

[3] correlation between AP and TP

[4] correlation between WHC and WRE

AP: aeration porosity (%); TP- total porosity (%); WHC- water-holding capacity (g ml^{-1}); WRE- water-retention efficiency (%); EC- electrical conductivity (mmhos•cm^{-1}); Expt- Experiment

Control 1: 100% long fiber sphagnum moss;

Control 2: 50% peat moss, 25% perlite, and 25% pine tree bark;

Control 3: 33.33% river pebbles, 33.33% tree fern root, and 33.33% wood charcoal;

Control 4: 66.67% sphagnum moss, and 33.33% tree fern root

CF: 50% coconut fiber; OF-25% osmunda fiber; WCC- 25% wood charcoal chips; CTF- 25% chopped tree fern root; CRH-25% charcoaled rice husk; CD-50% coconut coir dust; SM- 50% sphagnum moss

only T2 with CD and T3 with CD fall within the required WHC range while the WRE for all mixes are less than 80% (unsuitable). For porosity characteristics, AP and TP were found to be highly correlated. AP readings for all mixes were above 20 to 25% (suitable) while TP readings of all mixes except C2 and T1 with CD, T2 with CD, and T3 with CD are greater than the standard 60%. Potting

Table 5. Physico-chemical properties of individual organic substrates for plantlets of Nepenthes truncata Macf.			
Substrates	Water retention efficiency[1] (%)	Electrical conductivity (mmhos cm^{-1})	pH
Coconut coir dust	35.67b	0.27	7.1
Coconut fiber	6.67de	0.69	7.1
Charcoaled rice husk	38.33b	1.01	5.7
Osmunda fiber	2.67e	1.43	6.4
Peat moss	25.67c	0.32	4.9
Perlite	86.67a	0.14	7.1
Pine bark chips	88.67a	0.59	5.2
River pebbles	2.67e	0.11	8.0
Sphagnum moss	38.33b	0.96	6.8
Tree fern root	1.00e	0.34	5.5
Wood charcoal	3.00e	0.52	8.0
Mean	29.94	0.58	6.53
P value	0.00		

[1] means within column with common letter(s) or no letters are not significantly different using DMRT at alpha=0.05.

mixes with coconut coir dust tend to hold more water compared to the other mixes (Table 4).

Among the components (Table 5), wood charcoal and river pebbles may be too basic and not ideal to be used while peat moss, tree fern, charcoaled rice husk, and pine bark chips are acidic and may be suitable. Thus, treatments with wood charcoal or river pebbles have lower rates of survival than most of those with acidic components in Experiment 1. In terms of WRE, pine bark chips and perlite are significantly better compared to other components (Table 5). Thus, C2 potting mix was favorable for the survival of the plantlets in Experiments 1 and 2.

It seems that the combination of acidic reaction and intermediate WRE from sphagnum moss and charcoaled rice husk make an ideal potting mix as shown by high survival rates of both small and medium plantlets of T6 (Tables 3, 4) in Experiment 1. Based on physico-chemical properties, coir dust may be used as a substitute to sphagnum moss. However, beneficial microorganisms found in sphagnum moss that prevents root rot pathogens (Premier Press 2000), probably absent in coconut coir dust, may help in the establishment of *N. truncata* plantlets.

Conclusions

The organic substrates coir dust (CD) and sphagnum moss (SM) could be interchangeable, and both were superior to coconut fiber (CF) as a component for moisture retention, while charcoaled rice husk (CRH) was superior over wood charcoal chips (WCC) and chopped tree fern (CTF) as a component for aeration based on survival and leaf condition parameters. Both small and medium plantlets performed better in Treatment 6 (T6) (2:1:1 mixture of SM, OF, and CRH) and C1 (SM). Leaf condition was found to be significantly better in Treatment 5 (2:1:1 mixture of SM, OF, and CTF) and T6 compared to other mixes while small plantlets have significantly better leaf condition than medium plantlets. Physico-chemical properties of the superior treatment, T6, suggest that an appropriate medium for growing *Nepenthes truncata* should have the following characteristics: slightly acidic (pH=6.8), moderate electrical conductivity (0.85 mmhos·cm^{-1}), high aeration porosity (61.58%) and total porosity (77.72%), and moderate water-holding capacity (0.29 g·mL^{-1}) and water retention efficiency (23.30%).

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