



RESEARCH PAPER

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Variation of Palynomorphological and pollen production of some invasive plant species of Asteraceae family in conservation areas of tropical rain forest, West Sumatra, Indonesia

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Abstract

Some conservation areas in tropical forests of West Sumatra have been invaded by some invasive plants species from family Asteraceae. Characterization and pollen production of these plants are very useful to identify and clarify their invasiveness. This paper analyzed the ability of twelve invasive plants in producing the pollens, as determinant agents of reproductive biology. The results of the pollen analysis showed that the pollen number per flower among invasive plants species were varied from 389 ± 14 in *Tridax procumbens* L. to 3739 ± 105 in *Mikania micrantha* Kunth. Most of species studied (92%) were produced the high pollen numbers that implied the xenogamous pollination system while *T. procumbens* produced the small number of pollen. There were variations in shape, ornamentation, and aperture of the pollen of twelve invasive plants studied. Three types of pollen shape were detected, i.e. Oblate-spheroidal shape (*Ageratum conyzoides* (L.) L., *Crassocephalum crepidioides* (Benth.) S. Moore, *Elepanthopus mollis* L., *Elepanthopus tomentosus* L. and *T. procumbens*), spheroidal shape (*Clibadium surinamense* L.) and prolate-spheroidal shape (*Erigeron sumatrensis* Retz., *Austroeupatorium inulaefolium* (Kunth.) R. M. King. & H. Rob., *M. micrantha*, *Sphagneticola trilobata* (L.) Pruski, *Acmella paniculata* Wall. ex DC. R. K. Jansen and *Vernonia sinerea* (L.) Lex.. The pollen surface of all species studied covered with spines. Two types of ornamentation were detected in the invasive plant studied, i.e. lophate type in *E. mollis*, *E. tomentosus* and *V. sinerea* and echinate type in others nine invasive plant species studied.

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Introduction

The Sumatran tropical forests, especially in West Sumatra, have abundant plants biological resources. Those plants are very potential and important for sustainability utilization on community life. Recently, biodiversity in Sumatran tropical forests has become threatened due to natural and man-made disasters. The threat of invasive plants invasions in various conservation areas can decrease plants diversity that ultimately leads to adverse impacts on communities. Invasive plants have occurred in various habitats such as tourist destination places, agricultural lands near the forests, and national parks areas in the tropical forests. Invasive plants can reduce and replace the composition of native vegetation that can threaten biodiversity in the regions. The existence of invasive plants in various conservation areas of West Sumatra indicates that the invasive plants have been colonized in those regions (Syamsuardi *et al.*, 2006). Asteraceae is the most plants family that invades those conservation areas. Related to the invasiveness of a plant species, some species of the Asteraceae were included in the 100 most destructive species in the world (Lowe *et al.*, 2004).

Invasive plants in the natural can compete with local plants in forest community not only in space, nutrients but also in pollinators visiting. Invasive plants species can decrease the success of plant reproduction in natural plant communities (Dangremond, Pardini, and Knight, 2010; Molano-Flores, 2014; Goodell and Parker, 2017). The successful of sexual reproduction of a plant determines the conservation of plant species in a natural plant community. Therefore, pollen is a unique plant material that plays a role in sexual reproduction and also conservation of a plant species. The relationship between the type of pollen ornamentation and the pollination system was revealed by a particular plant taxonomic group (Sannier *et al.* 2009). The patterns of pollen ornamentation in Angiosperm plants were so conservative, so they have been used in the identification and classification of family Asteraceae (Robinson and Skvarla, 2014).

A specific pattern of ornamentation was also applied to detect the presence of allegedly causing pollen from *Ambrosia artemisiifolia* (Asteraceae), an invasive plant (Peternel, Milanovic and Srnec, 2008). Recently, the populations some species of Asteraceae have been colonizing in the tropical forests of Sumatra. Thus, analysis of the palynomorphological and pollen reproduction of these plants may provide understanding the invasiveness the species studied. This study was important to identify and manage for controlling the invasive plant species in conservation areas of tropical forest West Sumatra.

Materials and methods

Study areas

Twelve recorded invasive plants from Asteraceae (*A. paniculata* = AP, *A. conyzoides* = AC, *A. inulaefolium* (Kunth. = AI, *C. surinamense* = CS, *C. crepidioides* = CC, *E. mollis* = EM, *E. tomentosus* = ET, *E. sumatrensis* = ES, *M. micrantha* = MM, *T. procumbens* = TP, *S. trilobata* = ST and *V. sinerea* = VS) were collected from School Forest of Universitas Andalas, Limau Manis, Padang and Solok Botanical Gardens Solok City, West Sumatra, Indonesia from January-April 2017. Samples were processed to Herbarium Specimen and deposited in Herbarium Universitas Andalas as the voucher specimens of this research.

Data Collection

The flowers of twelve collected samples were incubated in FAA (formaldehyde acetic acid) preserve solution. Pollen grains were analyzed at Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas. The some parameters of micromorphology pollen grains (the size, polarity, shape, ornamentation and aperture) were examined using scanning electron microscope (SEM) at Laboratory of Widiasatwa Loka LIPI Cibinong Bogor, Indonesia The number of pollen were examined using two techniques, i.e. directly examination if the pollen number was lesser than 1000 grains per theca. In case of the pollen number per theca more than 1000 grains, the pollens were counted with the dilution technique.

Pollens were stained using lacto-phenol cotton blue solution. Analysis micromorphology pollen grains using scanning electron microscope (SEM) were conducted at Laboratory of Widyasatwa Loka LIPI Cibinong Bogor, Indonesia

Data analysis

The Data of micromorphological pollen of twelve invasive plant species were analyzed with describing the size, aperture, shape, and pollen ornamentation types based on observation under microscope and photograph of SEM. The terminology used of characteristic micromorphology pollens were referred to Halbritter *et al.* (2008). The different number of pollen per flower from twelve invasive plant species were presented in comparison graph between the species studied. Based on number of the pollen, the breeding system of studied species was presumed (Cruden, 1977).

Results and discussion

Observation pollen grains from twelve invasive plants indicated that all unit types are monad or monolete. The polar axis showed the variation in length with the smallest one (P = 24.3 μm) in *A. paniculata*, while the longest one (P = 34.2 μm) was detected in *E. mollis*

and *E. tomentosus*. Pollen grains of *Acmella paniculata* had also the smallest value of equatorial axis length (E = 22.1 μm) while the largest one was detected in *E. mollis* and *E. tomentosus* (E = 35.5 μm). Based on measurement of the equatorial length, the size of the pollen was clarified. Three species (*A. paniculata*, *E. sumatrensis*, and *M. micrantha*) had the small pollen (22.1 - 23.3 μm), while nine others species had the medium size of pollen (25-35.5 μm). Furthermore the value of P/E ratio determines the shape of pollen grains examined.

The smallest P/E value was found in *E. mollis* and *E. tomentosus* (P/E = 0.96) whereas the longest one (P/E = 1.33) was found in *S. trilobata* (Table 1). Based on the P/E values, the pollen grains of twelve species of Asteraceae family were distinguished into prolate-spheroidal, spheroidal and oblate-spheroidal shape. The prolate-spheroidal shape was found in *A. paniculata*, *A. inulaefolium*, *E. sumatrensis*, *M. micrantha*, *S. trilobata*, and *V. sinerea*. The spheroidal shape was observed in *C. surinamense*. In case of the oblate-spheroidal shape was found in *Ageratum conyzoides*, *C. crepdioides*, *E. mollis*, *E. tomentosus* and *T. procumbens* (see Fig. 1A and 1B).

Table 1. Comparison of size, shape, ornamentation and type of aperture of twelve invasive plant species from Asteraceae family.

No.	Species*	Size um			Shape*	Ornamentation type	Aperture Type	Additional characters
		P	E	P/E				
1	AP	24.3	22.1	1.09	Prolate spheroidal	echinate	tetracolporate	long spines, bends pointed, narrow and solid
2	AC	30.3	30.5	0.99	Oblate spheroidal	echinate	tricolporate	The spines are long, erect, pointed narrow and rather dense
3	AI	27.1	25.2	1.07	Prolate spheroidal	echinate	tricolporate	short spikes, pointed. broad base, solid
4	CS	31.5	31.2	1.00	spheroidal	echinate	tricolporate	long spines, bent, pointed narrow and rather dense.
5	CC	27.0	27.3	0.99	oblate-spheroidal	echinate	colporate	short spikes. pointed. broad base. solid.
6	EM	34.2	35.5	0.96	Oblate-spheroidal	lophate	porate	very short spines attached to the ridge that surrounds many of the lacunar
7	ET	34.2	35.5	0.96	Oblate spheroidal	lophate	porate	short spines. attached to the thick ridge surrounding the lacunae.
8	ES	26.3	23.3	1.12	prolate-spheroidal	echinate	colporate	Spines are long. Irregular, crooked ends.
9	MM	26.3	23.3	1.12	Prolate-spheroidal	echinate	colporate	Spines are long. pointed. wide. sparse. slightly rare.
10	ST	32.5	28.7	1.33	prolate-spheroidal	echinate	colporate	Spines are long. tapered. wide and sparse.
11	TP	27.7	27.8	0.99	oblate-spheroidal	echinate	tricolporate	The spines are long. somewhat narrow. solid with pointed tips
12	VC	27.8	24.8	1.12	prolate-spheroidal	lophate	porate	Longer, sharp spikes attached to the thick ridges. encircling much narrower lacunar

*The meaning of abbreviation were presented in material and method; terminology according to Wortley *et al.* (2008).

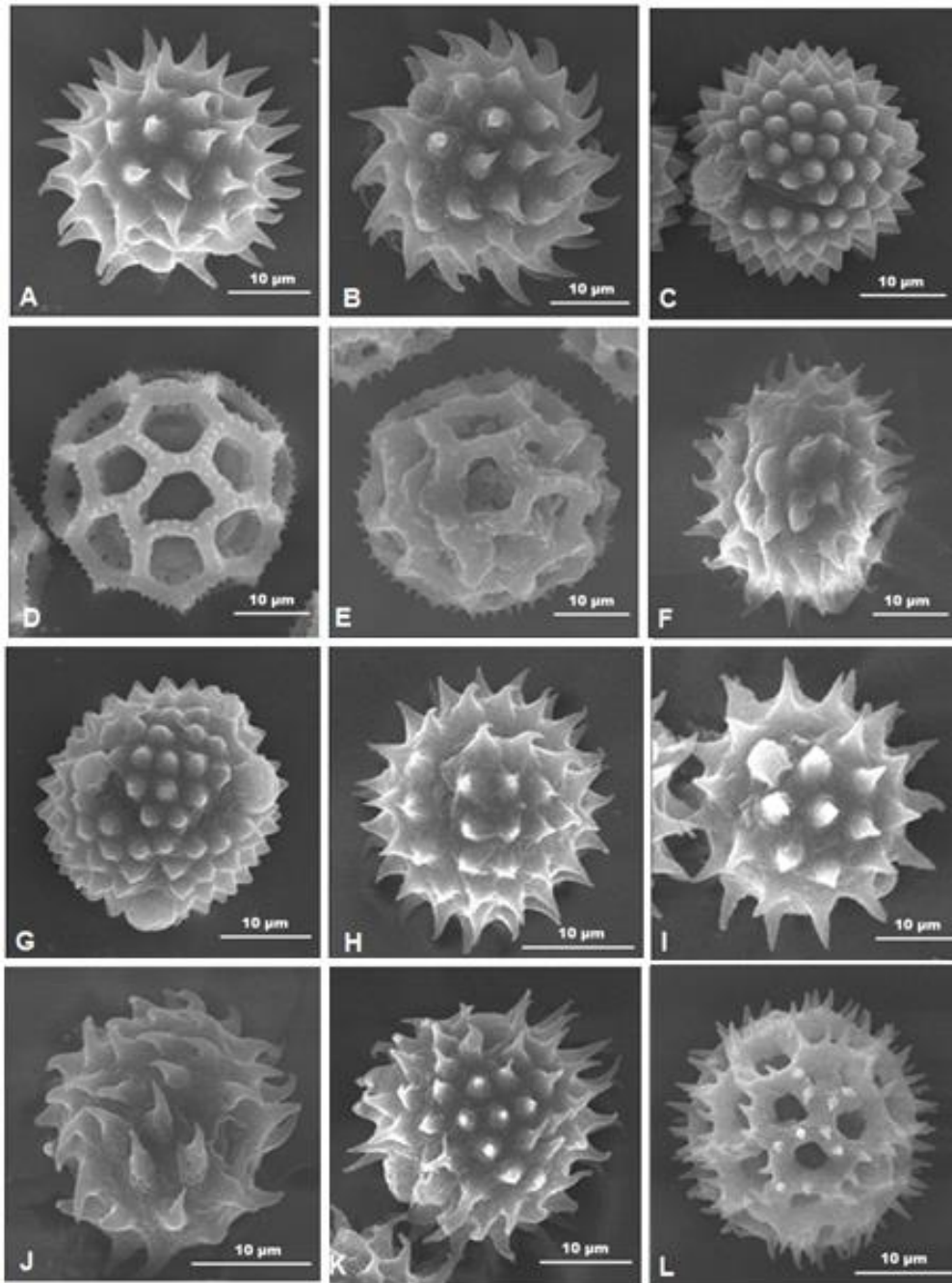


Fig. 1. The shape of pollen under SEM analyzed, *A. conyzoides* (A); *C. surinamense* (B); *C. crepidioides* (C); *E. mollis* (D); *E. tomentosus* (E); and *E. sumatrensis* (F). *A. inulaefolium* (G); *M. micrantha* (H); *S. trilobata* (I); *A. paniculata* (J); *T. procumbens* (K); and *V. cinerea* (L).

Furthermore, examination the surface of pollen grain indicated that all the surface pollen grains covered by spines with the variation in its length and sharpness. Two types of ornamentation of pollen grain were

clarified in twelve invasive plants i.e. echinate and lophate-echinate type. The former type was the pollen had the the outer surface with many spines, these type was detected at *A. paniculata*, *A. conyzoides*, *A. inulaefolium*, *C. crepidioides*, *C. surinamense*, *E. sumatrensis*, *M. micrantha*, *S. trilobata* & *T. procumbent*, while the later type had some lacunae with spiny ridges around them. This type was observed in three species i.e. *E. mollis*, *E. tomentosus* and *V. sinerea*. Observation of the aperture of pollen grains of twelve species exhibited three different types

i.e. tetralcolporate type was detected in *A. paniculata*, tricolporate type in *C. crepidioides*, *E. sumatrensis*, *M. micrantha* and *S. trilobata*, and porate type in *Elepanthopus molis*, *E. tomentosus* and *V. sinerea*.

Examination of pollen grain twelve invasive plants exhibited the variation in number pollen grain per flower with the highest mean number was found in *M. micrantha* (3739 pollen) followed by *A. paniculata* (2796), *S. trilobata* (2768 pollen), *C. surinamense* (1872 pollen), and so on, while the lowest number of pollen was detected in *E. tomentosus* (389 pollen) (See Fig. 1).

Moreover, the high variation of twelve invasive plants in producing pollen grains implied their success of the reproductive biology. Cross-pollination usually occurs in plants that have many flowers, numerous pollen and many seeds (Wyatt, 1983; Ghazoul 2005; Yang *et al.* 2017). The numbers of pollens was an important aspect to determine the success of pollination. The plant with a large number of pollen production indicated its ability to cross-pollination or xenogamy (Cruden, 1977; Philbrick and Riesberg 1994). In contrast, plants with a little pollen tend to do the self-pollination (autogamy). The huge number of pollen in *M. micrantha* proved that this plant was one of the most harmful invasive species in the world (Lowe *et al.*, 2004). In addition, the most numerous of propagules production implied that invasive plants had highest ability to colonize the conservation areas compared to the others of invasive plants in Asteraceae groups. To increase population size and distribution expanse, the invasive plants maintain the efficient reproductive strategies (Huang *et al.*, 2012; Hodač *et al.*, 2016).

The characteristic of pollen grain (number, dispersal unit, polarity, size, shape, and structure) of flowering plants have been analyzing for various purposes. The Palynological study had been conducted to classify the taxa and to analyze phylogenetic relationship between the taxonomic groups. For classification of grass, the characteristic surface, ornamentation, and texture of pollen were important to examined (Mander *et al.*, 2013) and evolution in Asteraceae

(Barreda *et al.*, 2015; de Souza *et al.*, 2015; Wortley *et al.*, 2008; 2012; Robinson and Skvarla, 2014). Pollen grains of most of the species of the Asteraceae was unique the echinate type and differed from the pollen of other families by the relatively small in size and the spines with the irregular arrangement (Adekanmbi, 2009). Concordance to results of this study, 75 % (nine of twelve) of species studied presented the echinate type of ornamentation pollen. Concerning to the level of the invasiveness of twelve species studied, *M. micrantha* have been noted as one of 100 species most invasive in the world (Lowe, 2004) and now invade of the conservation area in West Sumatra. Based on its characteristic ornamentation pollen grain, it can be distinguished to those others invasive species by echinate type, regular, flat, long spine with acuminate, and bent tip (Fig. 1H).

Furthermore, there was a correlation between ornamentation of pollen grain with pollinator type. Wind or water pollination had the smooth pollen while biotic pollination had the sculptured pollen (Sannier *et al.*, 2009). This fact suggested that all of the invasive plants studied maintain their reproduction through biotic especially insect pollination.

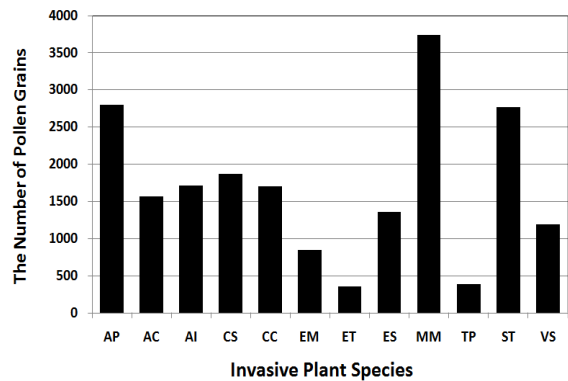


Fig. 2. The number of pollen per flower of twelve invasive plants in conservation areas in West Sumatra.

Moreover, the identification of invasive plants very important to protect the conservation area from the invasion of alien plant species. The characteristic of pollen grain can be additional evidence to classify and clarify invasive species in the natural plant community. There was variation in shape, ornamentation, and aperture of twelve invasive plants studied (Table 1).

The differentiation of pollen grain characteristics between species in Asteraceae related to taxonomic group (Wortley *et al.* 2008). Variation in lophate and sublophate type was exhibited in tribe Vernonieae, Asteraceae (Robinson and Skvarla, 2014). Based on this study, three species i.e. *E. molis*, *E. tomentosus* and *V. sinerea* had the lophate type but others nine species had the echinate type of ornamentation pollen grain. Wortley *et al.* (2008) explained that echinate was the pollen with spines on the surface at least 1 μm in height. In case of lophate was the pollen having lacunae that surrounding by ridges.

Finally, we concluded that there were variations of shape, ornamentation, and aperture of the pollen of twelve invasive plants studied. Three type of pollen grains shape were detected, ornamentation of twelve invasive plants studied was echinate type. Three of twelve invasive species had the lophate type of pollen surface and nine species with non lophate type. These characteristic of micromorphology pollen were useful to identify the occupied invasive species of Asteraceae and control their invasion in conservation areas of tropical West Sumatra Forest. The high variation in producing pollen grains of twelve invasive plants implied their success of the reproductive biology and degree of their invasiveness. Cross-pollination usually occurs in plants that have many flowers, numerous pollens, and many seeds. Based on the number of pollen species studied suggested that they were maintained xenogamy breeding system, and *M. micrantha* was a species with the more invasive than other species studied and the lowest number of pollen in *E. tomentosus* implied the less invasive than others species studied.

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