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Leaf Surface Characteristics of Selected Malaysian Weed Species of Oil Palm

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ABSTRACT

Laboratory and glasshouse studies were conducted to examine the leaf surface characteristics of selected weed species of oil palm. The broadleaf weeds selected were *Asystasia gangetica, Borreria latifolia, Cleome rutidosperma, Clidemia hirta, Diodia ocimifolia* and *Mikania micrantha,* while for the narrow leafs, *Axonopus compressus, Cyperus kylingia, Eleusine indica, Paspalum conjugatum* and *Pennisetum polistachyon* were investigated. The weeds were categorized into different types of roughness based on the macroscopic roughness, microscopic roughness and the estimation of three roughness parameters: Ra (arithmetic average height parameter), Rq (root-mean-square roughness parameter, corresponding to Ra), and Rz (average of high peaks and low valleys over the evaluation length). The leaf was examined using scanning electron microscopy (SEM) for the surface roughness, while the epicuticular wax content of the leaf was extracted using chloroform. The amount of wax extracted from the weeds varied between species. For broadleaf plants, *Mikania micrantha* (44.22 $\mu g/cm^2$) was identified as the plant that contained the highest quantity of wax. *Clidemia hirta* (24.03 $\mu g/cm^2$) and *Asystasia gangetica* (23.03 $\mu g/cm^2$) were grouped in the plants with a medium quantity of wax while *Cleome rutidosperma* (16.52 $\mu g/cm^2$), *Borreria latifolia* (14.19 $\mu g/cm^2$) and *Diodia ocimifolia* (10.75 $\mu g/cm^2$) were grouped in the plants with a low quantity of cuticular wax weight. For narrow leaf plants, *Eleusine indica* (44.23 $\mu g/cm^2$) and *Imperata cylindrica* (49.88 $\mu g/cm^2$) were recognized as the plants that contained a high quantity of wax. *Pennisetum polystachion* (32.16 $\mu g/cm^2$) and *Cyperus kylingia* (22.85 $\mu g/cm^2$) were categorized under the plants with a medium quantity of wax, whereas *Paspalum conjugatum* (19.59 $\mu g/cm^2$) and *Axonopus compressus* (16.78 $\mu g/cm^2$) were classified under the plant with a low quantity of wax. The wax on the abaxial surface data of the broadleaf weeds was found to be significantly different when compared to the adaxial surface data. In contrast, the amount of wax on the abaxial and adaxial leaf surface of the narrow leaf weeds was more or less similar. For the leaf surface roughness of the broadleaf species, *Borreria latifolia* was categorized as the roughest, followed by *Clidemia hirta, Diodia ocimifolia, Asystasia gangetica* and *Cleome rutidosperma. Mikania micrantha* had the smoothest leaf surface among the broadleaf species. On the other hand, the narrow leaf of *Pennisetum polistachyon* was identified as the roughest, followed by *Imperata cylindrica* and *Paspalum conjugatum,* while *Eleusine indica, Axonopus compressus* and *Cyperus kylingia* were categorized as having the smoothest leaf surface.

Keywords : Leaf surface characteristic, roughness, wax amount

ABSTRAK

Kajian makmal dan rumah kaca telah dilakukan untuk mengkaji kriteria permukaan daun rumpai terpilih dari ladang kelapa sawit. Rumpai daun lebar yang dipilih ialah *Diodia ocimifolia, Borreria latifolia,* *Clidemia hirta, Cleome rutidosperma, Mikania micrantha* dan *Asystasia gangetica*, sementara rumpai daun tirus yang dikaji ialah *Eleusine indica, Cyperus kylingia, Axonopus compressus, Pennisetum polistachyon* dan *Paspalum conjugatum*. Rumpai telah dikategori berdasarkan kepada kekasaran makroskopik, kekasaran mikroskopik dan parameter anggaran kekasaran: Ra (parameter purata ketinggian aritmetik), Rq (punca kuasa dua nilai parameter kekasaran, bersepadanan dengan Ra), dan Rz (purata tinggi dan rendah puncak lembah ke atas nilai panjang). Daun telah diperiksa dengan menggunakan mikroskopi imbasan elektron (SEM) untuk menilai kekasaran permukaan, sementara lilin epikutikular daun diekstrak dengan menggunakan kloroform. Kandungan lilin yang diekstrak daripada rumpai adalah bervariasi di antara spesis. Untuk tumbuhan daun lebar, *Mikania micrantha* (44.22 $\mu g/cm^2$) telah dikenalpasti sebagai tumbuhan yang mengandungi jumlah lilin tertinggi. *Clidemia hirta* (24.03 $\mu g/cm^2$) dan *Asystasia gangetica* (23.03 $\mu g/cm^2$) dikategorikan sebagai tumbuhan dengan jumlah lilin yang sederhana, sementara *Cleome rutidosperma* (16.52 $\mu g/cm^2$), *Borreria latifolia* (14.19 $\mu g/cm^2$) dan *Diodia ocimifolia* (10.75 $\mu g/cm^2$) dikategorikan sebagai tumbuhan dengan kuantiti lilin kutikular yang rendah. Untuk tumbuhan daun tirus, *Eleusine indica* (44.23 $\mu g/cm^2$) dan *Imperata cylindrica* (49.88 $\mu g/cm^2$) diakui mempunyai jumlah lilin terbanyak. *Pennisetum polystachion* (32.16 $\mu g/cm^2$) dan *Cyperus kylingia* (22.85 $\mu g/cm^2$) dikategorikan sebagai tumbuhan yang mempunyai jumlah lilin sederhana, manakala *Paspalum conjugatum* (19.59 $\mu g/cm^2$) dan *Axonopus compressus* (16.78 $\mu g/cm^2$) berada dalam kategori tumbuhan yang mempunyai jumlah lilin yang rendah. Jumlah lilin pada permukaan atas dan bawah daun bagi tumbuhan berdaun lebar mempunyai perbezaan yang nyata. Sebaliknya, jumlah lilin pada permukaan atas dan bawah bagi rumpai daun tirus adalah hampir bersamaan. Untuk nilai kekasaran permukaan daun daripada tumbuhan berdaun lebar, *Borreria latifolia* dikategorikan sebagai paling kasar diikuti oleh *Clidemia hirta, Diodia ocimifolia, Asystasia gangetica* dan *Cleome rutidosperma. Mikania micrantha* mempunyai permukaan daun yang paling halus di antara spesis daun lebar. Bagi tumbuhan berdaun tirus, *Pennisetum polistachyon* telah dikenalpasti sebagai paling kasar, diikuti oleh *Imperata cylindrica* dan *Paspalum conjugatum,* sementara *Eleusine indica, Axonopus compressus* dan *Cyperus kylingia* dikategorikan sebagai mempunyai permukaan daun yang halus.

Kata kunci: Ciri permukaan daun, kekasaran, jumlah lilin

INTRODUCTION

The activity of foliage applied herbicide must ultimately depend on the concentration of active ingredient that reaches the sites of action, together with the effect of the herbicide on the biochemical mechanisms that take place at these sites. The micro structures of leaf surface are among the factors that influence droplet retention. The amount of herbicide deposited on a weed canopy during spray application is influenced by many factors; such as plant morphology, spraying technique and herbicide formulation. Plant cuticles represent the interface between the plant and their environment, and are covered with a protective wax layer. The two major functions of the cuticle are to protect plants from an uncontrolled loss of water (Schreiber *et al*., 2001) and to reduce leaching of organic and inorganic substances from the leaf interior (Schonherr *et al*., 2000). Leaf waxes have been shown to be largely responsible for these barrier properties. The studies on leaf surface characteristics are crucial for understanding the effect of leaf surfaces on the effectiveness of herbicides applied to the plant. Leaf surfaces exhibit a great number of structural types (Baker and Parsons, 1971; Holloway and Baker, 1974; Barthlott, 1981, 1990). Holloway *et al.* (1976) defined three types of surface roughness dependent on the magnification needed to reveal different leaf characteristics in detail *i.e.* macroscopic, microscopic and ultra-micro roughness. This characteristic was important in order to study the surface of leaves such as corrugation, roughness, the presence of hairs or trichomes and the physiochemicals properties of the epicuticular wax. The objective of this paper was to determine the leaf wax and leaf surface roughness on selected noxious Malaysian oil palm weed species.

MATERIALS AND METHODS

Plants and Materials

Weeds were planted in a glasshouse at 34 ± 5 ºC during the day and 28 ± 3 ºC at night, with a 12 hour photoperiod. Relative humidity oscillated a few percent around 53%. The narrow leaved weeds used were *Imperata cylindrica, Eleusine indica, Paspalum conjugatum, Pennisetum polistachyon, Axonopus compressus* and *Cyperus kylingia,* while the broad leaved weeds namely *Asystasia gangetica, Borreria latifolia, Diodia ocimifolia, Mikania micrantha, Cleome rutidosperma* and *Clidemia hirta* were used in the experiment. The rhizomes of *Imperata cylindrica* and *Pennisetum polistachyon* were collected from Lembah Bidong, Setiu, Terengganu, Malaysia. The seeds of *Eleusine indica* and *Paspalum conjugatum,* and the seedlings of *Axonopus compressus* and *Cyperus kylingia* were harvested in a vegetable plot, Ladang 2 of Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. The seeds of *Asystasia gangetica, Borreria latifolia*, *Cleome rutidosperma* and *Diodia ocimifolia* were collected at the oil palm plantation of UPM, and stem cuttings of *Mikania micrantha* were obtained from Bukit Ekspo of UPM, Serdang, Selangor, Malaysia. The seeds of *Clidemia hirta* were collected from Ladang Rakyat Terengganu, Kuala Berang, Terengganu, Malaysia. Plants were watered daily and fertilized with NPK (15:15:15) fertilizer. Plants of 7 to 8 week old were used in this study. The first and second fully expanded leaves of 7 to 8 week old plants; located on the highest stem physically in broadleaf, or from coleoptile downward in narrow leaf, were harvested for the experiment. The intention was to uniformly sample leaves exposed to the average amount of sunlight and have a similar development stage.

Leaf Wax Extraction

The study on leaf wax was conducted by evaluating the amount of leaf waxes on both and different surfaces of leaf. Sixty leaves from each weed species were used in each experiment. Their weight and area were determined. To extract the wax for both surfaces of leaf, a combination technique of wax extraction was adopted from Jetter *et al.* (2000). The leaves were dipped in 25 mL of chloroform ($CHCl_3$, Merck, Darmstadt, Germany) for 60 s in scintillation vials. To extract the wax for abaxial or adaxial surface of leaf, the chloroform was first warmed in a 500 mL glass beaker placed in a water bath (AYELA SB-650) and maintained at 40 ^{o}C. Then, 2 mL of warm chloroform was slowly run on the 1 cm^2 leaf surface and collected in 25 mL scintillation vials.

The solvent was allowed to evaporate between applications of consecutive portions in a scintillation vial. The weights of the scintillation vials before and after the experiment were determined. The wax content was calculated by subtracting the initial weight and was expressed on the leaf area basis. For broadleaf plants such as *Cleome rutidosperma* and narrow leaf plants which had a small leaf area, a masking tape was attached on abaxial surface and *vice versa* to avoid chloroform from being detached on unwanted surface.

Leaf Surface Roughness

Selected dried leaves of all species of narrow leaf and broadleaf plants were soaked in tap water for 1 hr, and then halved along the midrib with a razor blade. Each specimen was wrapped around a wooden dowel (d = 20 mm) and dried. A rectangular section (8 mm x 20 mm) was cut from the curved portion of each halved leaf. An upper curve was mounted on a SEM stub (d = 32 mm) with the support of an aluminum stand. The stands allowed the curved leaf sections to sit on the stub at an angle that enabled a view of the horizontal section of the leaf surface. The specimens were mounted on a viewing stub with an adhesive tape and were then coated with gold (20-30 nm) in a sputter coater. The stub was then placed in the scanning electron microscope (JOEL JSM-5610LV). An electron gun potential of 5 kV was selected to reduce the electron damage to the specimen and a small final aperture on the optical column was used to obtain a good depth of focus (Kerns and Barlocher, 2008). The images of six samples of abaxial surface for each type of weed were captured to measure the leaf surface roughness.

Quantification and Qualification of Leaf Surface Roughness

The images captured were analyzed using the Adobe Photoshop CS2™ and Fovea Pro™ software for windows (Kerns and Barlocher, 2008). The images were calibrated for the magnification used, they were then converted to grayscale images and finally transformed into pure black and white images by using the bi-level threshold filter. Any area below the contour of the leaf surface structures was filled in with black and the background of the image was white. This protocol created a section profile for each leaf surface. The measurement of leaf surface roughness of each profile was done by using the IP* Surfaces filter by selecting the Section Profile option. The roughness parameters Ra, Rq and Rz were calculated in μm. Among all these parameters, Ra is most commonly used. Ra is the arithmetic average height parameter, or centre line average, and is defined as the average absolute deviation of the roughness irregularities from the mean line over one sampling length. It averages all peaks and valleys along the evaluation length, giving a general description of the surface, and neutralizes outlaying points. Rz is the average absolute sum of the five highest peaks and the five lowest valleys over the evaluation length, and it puts more emphasis on high peaks and low valleys. Rq is the root-mean-square roughness parameter corresponding to Ra. It represents the standard deviation of the distribution of surface heights and is more sensitive than Ra to large deviations from the mean line (Gadelmawla *et al.,* 2002).

Two types of surface roughness dependent on the magnification were used to reveal different leaf characteristics in detail. First is macroscopic roughness which is visible to the naked eyes, or under magnifications of up to 60 times. The second is microscopic roughness which is visible under magnifications of *ca.* 120x (Holloway *et al.*, 1976). For the study of macroscopic roughness, leaf hair or trichome length, frequency and distribution were calculated based on 1 cm^2 of leaf area surface. To measure the microscopic roughness, the size and shape of the outer surfaces of epidermal cells were determined. The size of epidermal cell can actually determine the geometry of the grooves between cells. These grooves are the most important feature in determining the microscopic roughness.

Data Analysis

Six replicates were made for each treatment and the mean values were determined. All experiments were conducted in a complete randomized design (CRD). The data obtained were subjected to analysis of variance (SAS) and the mean values were compared by Tukey's Honestly Significant Difference (HSD) Test. In the study of wax amount on different surfaces, the mean value was analyzed using T-test in order to compare the significant differences of wax amount on abaxial and adaxial leaf surfaces.

RESULTS AND DISCUSSION

Mass of Wax for Both Surfaces of Leaf

The mean comparison of wax weight varies among broadleaf plants. The wax weight of *Clidemia hirta* and *Asystasia intrusa* is nearly similar. The analysis of variance also showed no significant difference of mean comparison between the wax weight of *Cleome rutidosperma, Borreria latifolia* and *Diodia ocimifolia.* The species of *Mikania micrantha* (44.22 μg/cm^2) was recognized as a plant with the highest amount of wax. *Clidemia hirta* (24.03 μg/cm^2) and *Asystasia gangetica* (23.03 μg/cm^2) were grouped in plants with medium amounts of wax while, *Cleome rutidosperma* (16.52 μg/cm^2), *Borreria latifolia* (14.19 μg/cm^2) and *Diodia ocimifolia* (10.75 μg/cm^2) were grouped in plants with a low amount of cuticular wax weight. Table 1 demonstrates the mean leaf weight (g), leaf area (cm^2) and cuticular wax weight (μg/cm^2) of the broadleaf plants.

Table 1. Means of cuticular wax, leaf weight and leaf area of broadleaf species.

Weed species	Mean leaf weight (g)	Mean leaf area (cm^2)	Mean cuticular wax ($\mu g/cm^2$)
		*Mean ± (S. E.)	
Mikania micrantha	0.32 ± (0.04)^{bc}	16.04 ± (1.77)^{a}	44.22 ± (2.41)^{a}
Clidemia hirta	0.28 ± (0.02)^{bc}	15.31 ± (0.95)^{a}	24.03 ± (1.73)^{b}
Asystasia gangetica	0.24 ± (0.04)^{c}	11.23 ± (0.94)^{c}	23.03 ± (2.03)^{b}
Cleome rutidosperma	0.41 ± (0.02)^{a}	13.97 ± (0.94)^{b}	16.52 ± (4.21)^{c}
Borreria latifolia	0.36 ± (0.03)^{ab}	16.03 ± (0.81)^{a}	14.19 ± (2.02)^{c}
Diodia ocimifolia	0.11 ± (0.03)^{d}	4.88 ± (0.42)^{d}	10.75 ± (1.23)^{c}

Note: *Means within columns followed by same letter are not significantly different ($P \leq 0.01$) (Tukey's)

In an experiment on six species of narrow leaf plants, the results showed that mean comparison of wax weight varies among species. From the data, the wax weight of *Eleusine indica* (44.23 $\mu g/cm^2$) and *Imperata cylindrica* (49.88 $\mu g/cm^2$) showed no significant difference and are recognized as plants with a high amount of wax weight. *Pennisetum polystachyon* (32.16 $\mu g/cm^2$) and *Cyperus kylingia* (22.85 $\mu g/cm^2$) were grouped in medium amount of plant wax, while *Paspalum conjugatum* (19.59 $\mu g/cm^2$) and *Axonopus compressus* (16.78 $\mu g/cm^2$) were grouped in plants with a low amount of wax. Table 2 shows the mean of leaf area, leaf weight and cuticular wax weight for the species of the narrow leaf plants.

Table 2. Means of cuticular wax, leaf weight and leaf area of narrow leaf species.

Weed species	Mean leaf weight (g)	Mean leaf area (cm^2)	Mean cuticular wax ($\mu g/cm^2$)
		*Mean ± (S. E.)	
Imperata cylindrica	0.40 ± (0.03)^{a}	16.34 ± (0.87)^{a}	49.89 ± (2.41)^{a}
Pennisetum polistachyon	0.36 ± (0.02)^{a}	15.91 ± (0.75)^{a}	33.13 ± (1.33)^{b}
Cyperus kylingia	0.21 ± (0.04)^{b}	11.23 ± (0.94)^{c}	22.85 ± (0.83)^{c}
Paspalum conjugatum	0.14 ± (0.03)^{b}	8.12 ± (1.94)^{d}	19.59 ± (3.23)^{c}
Axonopus compressus	0.19 ± (0.03)^{b}	14.03 ± (0.62)^{b}	16.79 ± (3.02)^{c}
Eleusine indica	0.15 ± (0.03)^{b}	4.82 ± (0.42)^{e}	44.24 ± (2.23)^{a}

Note: *Means within columns followed by same letter are not significantly different ($P \leq 0.01$) (Tukey's)

The amount of leaf wax varied among species. There are many factors that influence the development of cuticular wax on leaf plants such as air humidity, temperature, light density, water status and drought stress (Koch *et al.*, 2004; Jha *et al.*, 2008). Although all plants that were used in this treatment were planted in the same glasshouse and watered with a nearly similar amount of water, there was however no guarantee to confirm that the development of cuticular wax was the same among species.

McWhorther (1993) has reported that the cuticular wax weights per unit surface area were inversely related to fresh weight and surface area of leaves as well as the plant age. Wax weights are usually much higher when plants initiate new growth than later in the growing season (Freeman *et al.*, 1979; Baker and Procopiou, 1980; McWhorther *et al.*, 1990). Therefore, the cuticular wax weight for all species of broadleaf and narrow leaf plants in the present study were within the range of that found in the McWhorther study.

Mass of Wax for Different Surfaces of Leaf

The mean comparison was done by using T-test to compare the difference of wax weight on abaxial and adaxial surfaces for narrow leaf and broadleaf plants. The results showed that there was no significant difference in the amount of wax on abaxial and adaxial surfaces for narrow leaf plants. While for broadleaf plants, the results have shown some significant differences in the amount of wax on abaxial and adaxial leaf surfaces. The mean comparison of wax for abaxial and adaxial surfaces on the selected weed species are as shown in Table 3.

Table 3. Means of wax for abaxial and adaxial surfaces on the selected weed species.

	Leaf surface	
	Abaxial	Adaxial
Weed species	*Mean wax weight (μg/cm^2) ± (S. E.)	
Diodia ocimifolia	6.81 ± (1.35)[a]	3.89 ± (0.49)[b]
Asystasia gangetica	10.25 ± (1.08)[a]	6.29 ± (2.67)[b]
Borreria latifolia	7.28 ± (2.33)[a]	4.27 ± (1.09)[b]
Mikania micrantha	26.77 ± (2.69)[a]	17.13 ± (2.67)[b]
Clidemia hirta	14.88 ± (3.39)[a]	7.52 ± (3.61)[b]
Cleome rutidosperma	9.81 ± (2.97)[a]	4.53 ± (2.16)[b]
Paspalum conjugatum	9.11 ± (3.97)[a]	8.95 ± (1.79)[a]
Imperata cylindrica	24.00 ± (4.51)[a]	23.82 ± (0.89)[a]
Eleusine indica	18.78 ± (1.23)[a]	17.98 ± (3.67)[a]
Cyperus kylingia	9.78 ± (2.62)[a]	8.88 ± (1.15)[a]
Axonopus compressus	7.44 ± (1.69)[a]	8.01 ± (2.69)[a]
Paspalum conjugatum	8.52 ± (1.78)[a]	7.93 ± (1.48)[a]

Note: *Means within columns followed by the same letter are not significantly different ($P \leq 0.01$) (T-Test)

The difference of the wax concentration on leaf surfaces was influenced by many factors such as the plant morphology and the environment itself. The density of light received by the leaf may influence the development of wax amount on different surfaces. The leaf area of narrow leaf plants is smaller than the broadleaf plant, that makes the distribution of light density received by narrow leaf plants equal, but not for the broadleaf plant which has a bigger leaf area. Thus, the density of light is the main factor for leaf plants to build wax. The increase of light density during cultivation increased the total amount of cuticular wax per leaf area (Whitecross and Amstrong, 1972; Baker, 1974; Koch *et al*., 2004). The most important factor in determining the densities of light received by the leaf is the leaf arrangement on the plant. The arrangement of plants was a factor to indicate whether the leaves will receive the light densities equally or not for abaxial and adaxial surfaces.

The microclimate in which a plant grows can have a pronounced effect on cuticular wax content. The physical differences of leaf surface may be due to the changes of climate and environment even though all species of weeds were planted in the same glasshouse (Cowlishaw *et al*., 1984; Kirkwood, 1987; Steven and Schneider, 2004).

Quantification and Qualification of Leaf Surface Roughness

Considerable variability in surface roughness between all of the leaf plant species was apparent in both scanning electron microscopy (SEM) images and statistical descriptors. A visual assessment was used to qualify the leaf surface roughness and the statistical roughness descriptors were obtained to quantify the degree of surface roughness. The visual assessment of leaf surface roughness was done by evaluating the macroscopic and microscopic roughness. The length, frequency and distribution of leaf hair or trichome were calculated in order to determine the macroscopic roughness and the epidermal cell size was measured to qualify the microscopic roughness. The size and shape of the outer surfaces of epidermal cells determined the geometry of the grooves between cells. The bigger epidermal cell size means the less roughness of the surface. Comparative scales devised from the data of visual assessment agreed with the results of statistical roughness descriptors in order to categorize the leaf surface in their roughness categories.

From the analysis of broadleaf plants for the roughness parameters (Ra, Rq, Rz), the mean comparison varied among species. The Ra parameter showed the significant difference among *B. latifolia, C. hirta* and *D.ocimifolia* with other plants species ($P \leq 0.01$). The value of surface roughness for *C. rutidosperma* was nearly similar to *M. micrantha* and *A. gangetica*. Table 4 describes the value of surface roughness of broadleaf plants. *Borreria latifolia* was detected for having the roughest surface followed by *Clidemia hirta, Diodia ocimifolia, Asystasia gangetica* and *Cleome rutidosperma*. *Mikania micrantha* was identified for having the smoothest surface of leaf among all the broadleaf species evaluated in this study.

Table 4. Means of surface roughness.

	Roughness parameters *Mean (µm) ± (S. E.)		
Weed species	Rz	Ra	Rq
Mikania micrantha	171.10 ± (1.59)[e]	24.75 ± (1.01)[e]	32.93 ± (1.21)[e]
Clidemia hirta	498.97 ± (3.74)[b]	117.37 ± (3.11)[b]	145.01 ± (2.87)[b]
Asystasia gangetica	217.98 ± (6.29)[d]	41.12 ± (2.21)[d]	65.98 ± (1.48)[d]
Cleome rutidosperma	207.97 ± (0.91)[d]	25.52 ± (0.88)[de]	46.41 ± (2.29)[e]
Borreria latifolia	567.18 ± (4.26)[a]	158.03 ± (4.43)[a]	170.11 ± (2.25)[a]
Diodia ocimifolia	304.80 ± (1.01)[c]	90.10 ± (0.91)[c]	110.87 ± (1.73)[c]

Note: *Means within columns followed by same letter are not significantly different ($P \leq 0.01$) (Tukey's)

The roughness of adaxial surface of all species showed consistent differences, which largely conformed to SEM inspections (Table 5). Visually, the leaf of *Borreria latifolia* had the highest overall degree of surface complexity. Leaf hairs densely covered the entire adaxial surface with consistent arrangement. The adaxial surface of *Mikania micrantha* leaves were the least complex in surface roughness with a fewer number of trichome on the surface. The SEM images (Figures 1, 2, 3, 4, 5 and 6) showed the distribution of hairs on most broadleaf species excluding *Diodia ocimifolia* and *Mikania micrantha*. Both of the weed species have a distribution of trichome on the leaf surface but with different quantity.

Table 5. Distribution, number and length of hairs and trichome per mm² on the upper surface of mature leaves for broadleaf plants.

Weed species	No. hairs/mm²	Distribution on leaf	No. trichome/ mm²	Distribution on leaf	Length of hair/trichome (µm)
Asystasia gangetica	1 - 3	All over	0	Absent	243 - 397
Mikania micrantha	0	Absent	5 - 9	All over	25.5 - 39.9
Borreria latifolia	10 - 16	All over	0	Absent	441.9 - 571.5
Cleome rutidosperma	9 - 13	All over, but most on veins	0	Absent	149.3 - 226.7
Clidemia hirta	7 - 11	All over but especially around veins	0	Absent	875 - 1045
Diodia ocimifolia	0	Absent	20 - 25	All over	80 - 120

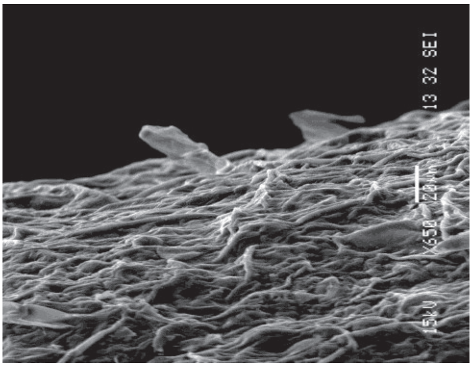

Fig. 1. Microsurface of *Mikania micrantha* adaxial leaf surface

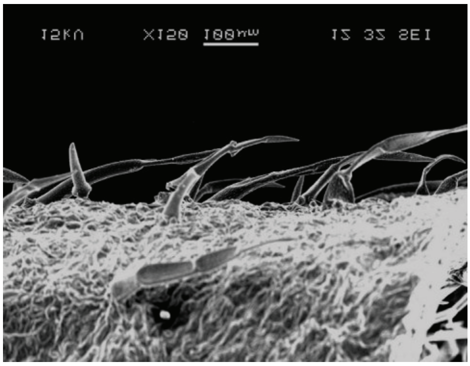

Fig. 2. Leaf hair density of *Borreria latifolia*

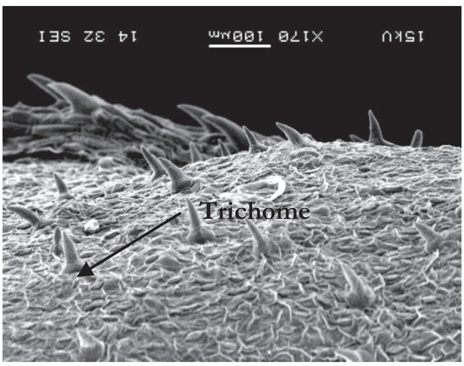

Fig. 3. The trichome distribution on *Diodia ocimifolia* leaf surface

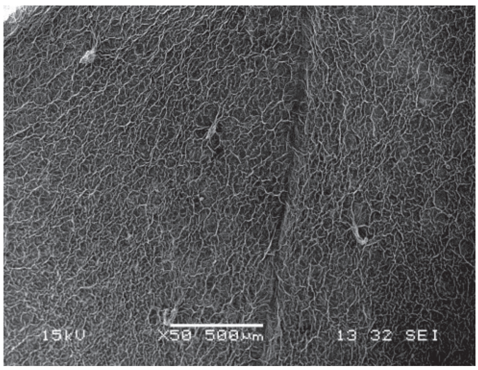

Fig. 4. Top view of microsurface of *Asystasia gangetica*

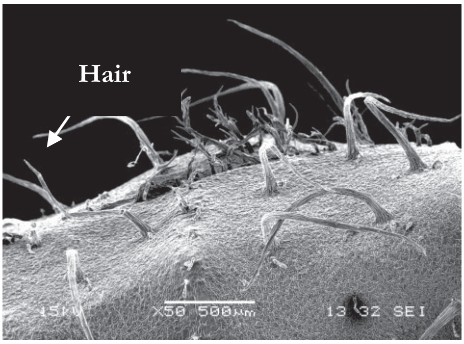

Fig. 5. The leaf hair distribution on *Clidemia hirta* leaf surface

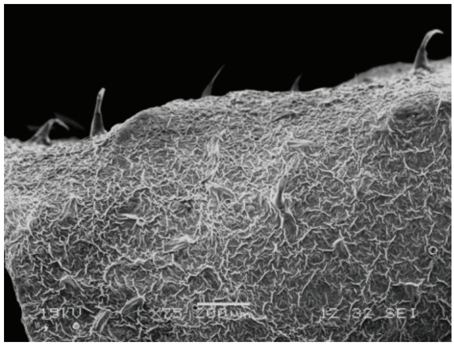

Fig. 6. The non-smooth microstructure of *Cleome rutidosperma* leaf surface

Table 6 describes the size of epidermal cells on mature leaves for broadleaf plants. The measurement of epidermal cell size on broadleaf plants showed *Asystasia gangetica* to have the biggest size of epidermal cells compared to other species, followed by *Cleome rutidosperma*, *Borreria latifolia, Diodia ocimifolia, Clidemia hirta* and *Mikania micrantha*. As mentioned previously, the cell size determines the geometry of the grooves between cells and the bigger size of epidermal cell means less roughness of the surface. However, the degree of surface roughness is also influenced by the distribution of hairs or trichome on the surface.

Table 6. Size of epidermal cells on mature leaves for broadleaf plants.

Weed species	Size of epidermal cells	
	Length (µm)	Width (µm)
Asystasia gangetica	40 - 55	40 - 55
Mikania micrantha	1 - 5	1 – 5
Borreria latifolia	30 - 60	10 - 30
Cleome rutidosperma	35 - 60	22 - 33
Clidemia hirta	22 - 46	2 – 17
Diodia ocimifolia	24 - 45	16 - 30

For the study of surface roughness on narrow leaf plants, the mean comparison of surface roughness parameter showed a significant difference among all species ($P \leq 0.001$). *Pennisetum polistachyon* have the roughest surface followed by *Imperata cylindrica, Paspalum conjugatum*, *Eleusine indica, Cyperus kylingia* and *Axonopus compressus*. Table 7 shows the value of surface roughness for narrow leaf plants.

Table 8 explains the distribution, number and length of hairs and trichome per mm^2 on the upper surface of mature leaves for narrow leaf plants. Image of SEM (JOEL JSM- 5610LV) for all narrow leaf plants showed the leaf of *Pennisetum polistachyon* (Figure 7) had the highest degree of surface complexity, followed by *Imperata cylindrica* (Figure 8) and *Paspalum conjugatum* (Figure 9). Leaf hairs densely covered the entire abaxial surface in consistent arrangement. Among all of the narrow leaf species, *Eleusine indica* (Figure 10), *Axonopus compressus* (Figure 11) and *Cyperus kylingia* (Figure 12) were categorized for having the smoothest surface due to their leaf surface characteristics. The abaxial surfaces of *Axonopus compressus, Cyperus kylingia* and *Eleusine indica* leaves were the least complex in surface roughness with no hairs or trichome found on the surface.

Table 7. Means of surface roughness parameter.

Weed species	Roughness parameters *Mean (μm) ± (S. E.)		
	Rz	Ra	Rq
Imperata cylindrica	423.95 ± (2.01)[c]	57.07 ± (1.39)[b]	93.23 ± (1.71)[b]
Pennisetum polistachyon	571.92 ± (5.01)[a]	223.87 ± (4.21)[a]	227.27 ± (2.08)[a]
Cyperus kylingia	110.15 ± (0.31)[d]	12.83 ± (1.00)[d]	21.18 ± (1.33)[d]
Paspalum conjugatum	433.81 ± (2.11)[b]	27.51 ± (0.89)[c]	45.12 ± (0.77)[c]
Axonopus compressus	45.38 ± (0.39)[f]	7.80 ± (1.35)[d]	10.07 ± (0.51)[e]
Eleusine indica	72.13 ± (0.78)[e]	14.86 ± (1.11)[d]	17.92 ± (1.84)[de]

Note: *Means within columns followed by same letter are not significantly different ($P \leq 0.01$) (Tukey's)

Table 8. Distribution, number and length of hairs and trichome per mm² on upper surface of mature leaves for narrow leaf plants.

Weed species	No. hairs/mm²	Distribution on leaf	No. trichome/mm²	Distribution on leaf	Length of hair/trichome (μm)
Eleusine indica	0	Absent	0	Absent	0
Imperta cylindrica	0	Absent	75 - 106	All over but especially on veins	19.4 - 47.2
Paspalum conjugatum	0	Absent	13 - 29	All over	16.6 - 35.6
Pennisetum polistachyon	10 - 18	All over	0	Absent	946.8 - 1371.4
Axonopus compressus	0	Absent	0	Absent	0
Cyperus kylingia	0	Absent	0	Absent	0

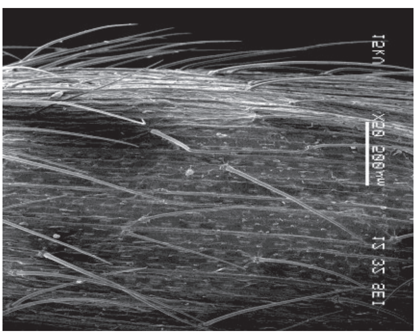

Fig. 7: The distribution of leaf hair on *Pennisetum polistachyon* leaf surface

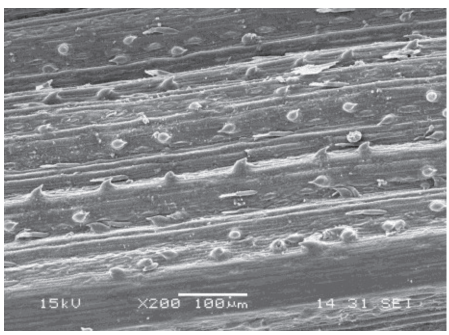

Fig. 8. The high density of trichome distribution on *Imperata cylindrica* leaf surface

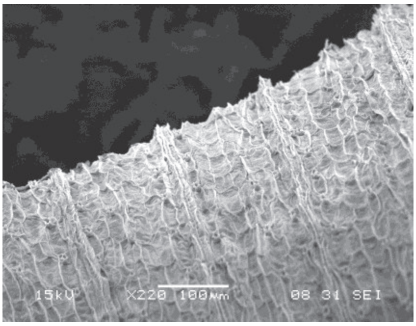

Fig. 9. The non-smooth *Paspalum Conjugatum* leaf microsurface

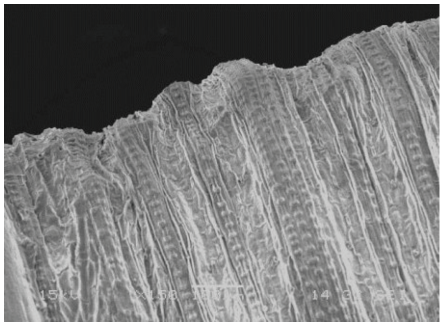

Fig. 10. The leaf surface microstruct of *Eleusine indica*

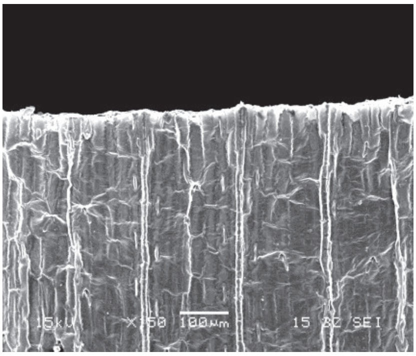

Fig. 11. The side view of *Axonopus Compressus* micro structure leaf

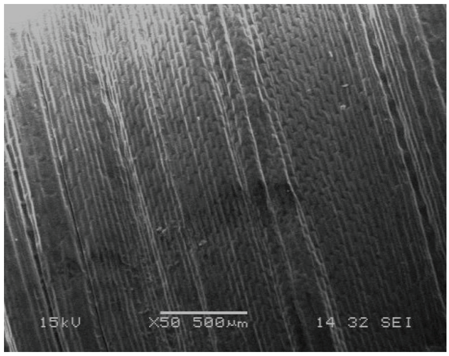

Fig. 12. The smooth microsurface of *Cyperus kylingia* leaf upper surface

The size of epidermal cells on narrow leaf plants is shown in Table 9. The measurement of epidermal cell size showed *Eleusine indica* to have the biggest size of epidermal cells followed by *Pennisetum polistachyon*, *Cyperus kylingia, Paspalum conjugatum, Axonopus compressus* and *Imperata cylindrica*. The data obtained confirmed the characteristics of the leaf surface.

Table 9. Size of epidermal cells on mature leaves for narrow leaf plants.

	Epidermal cell size	
Weed species	Length (µm)	Width (µm)
Eleusine indica	300 - 400	50 - 70
Imperata cylindrica	40 - 60	10 - 30
Paspalum conjugatum	75 - 95	15 - 35
Pennisetum polistachyon	121 - 156	23 - 47
Axonopus compressus	61 - 86	17 - 27
Cyperus kylingia	85 - 103	36 - 54

The type of leaf surface roughness is varied among the species of plants. The leaf surface roughness is not only determined by the morphology of plant species, but also influenced by the environmental factors as well as the development of epicuticular wax (Nevo *et al*., 2000). The leaf structure reflects the effect of water stress. The water availability was influenced by the stomata and trichome densities of the plants' leaf surface (Guerfal *et al*., 2009). The age of a leaf also influences surface roughness. As a leaf expands, hair density decreases and the grooves between the epidermal cells increase in size. The investigation of epidermal cell size on leaf surface revealed that the size of epidermal cells for narrow leaf plants is bigger than in the broadleaf plant. The size of epidermal cells was influenced by the pattern of veins on the leaf blade.

CONCLUSION

The study on leaf wax and surface roughness showed the variability of leaf surface characteristics among broadleaf and narrow leaf plants. The leaf surface characteristics of broadleaf plants are more complex compared to narrow leaf plants. The size of epidermal cells for narrow leaf plants was found to be bigger than the broadleaf plants. Types of leaf surface roughness and leaf wax amount varied among species. Leaf surface roughness is not only determined by the morphology of a plant species, but is also influenced by environmental factors as well as the development of epicuticular wax. These results gave some basic idea to the concept that the morphological characteristics of leaves of various weed species influence the behavior of herbicides on leaf surfaces which may lead to the differential activity of an herbicide from weed species to species, and can be optimized by using a specific surfactant.

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