# MORPHOLOGICAL TRAIT DIFFERENCE, GROWTH AND ECOPHYSIOLOGICAL PERFORMANCE OF MIKANIA MICRANTHA GROWN UNDER CONTRASTING LIGHT AND NUTRIENT REGIMES.

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# Abstract

*Mikania micrantha* , a world's worse weed, is rapidly expanding throughout the sub-tropical and tropical parts of Asian countries. Abundant growth and development of *Mikania*vines make them dominant over introduced habitat and causes significant damage to native floras, faunas and entire ecosystems. To investigate the role of environmental resources associated with its rapid growth and development, *Mikania* seedlings were grown at green house chamber by manipulating two levels of light and nutrient for 110 days. Total thirteen harvests had performedthroughout the study period on the weekly basis. Specific leaf area, leaf area ratio, leaf weight ratio, root weight ratio, relative growth rate, net assimilation rate and total biomass of seedlings grown under light and nutrient treatments were measured in each harvest. Photosynthetic performance of mature and fully grown *Mikania* seedlings was measured at 9th and 11th harvests. Mikania seedlings grown under full sun light and nutrient rich soil had allocated greater amount of total leaf area and total chlorophyll content capturedthe higher intensities of solar irradiances to attain maximum photosynthetic rate. Due to opportunistic capture and utilization of more resources for their physiological process and morphological allocation patternthe seedlings grown under full sun light with nutrient rich soil achievedcomparatively higher degree of net assimilation rate (NAR)and relative growth (RGR) than the seedlings grown at resource limited treatments. The seedlings grown under full sun light andnutrient rich soil had attained maximum biomass performance indicating the interaction effect between full light and high soil nutrient resources. *Mikania*seedlings grown under resource limited treatments had observed poor growth performance however these seedlings modified their morphological allocations such as higher specific leaf area and leaf area ratio to assimilatemore resources from the existing environment. The abilities of *Mikania* to tolerate adverse environmental conditions and efficiently utilize higher intensities of sun light and soil nutrient to increase overall performance and greater proportion of biomass allocation on aboveground parts make them to grow dominantly over the introduced habitat.



**Key words:** Chlorophyll, Dry weight, Invasive, *Mikaiamicrantha*, Net Assimilation Rate, Relative Growth Rate, Photosynthesis.

Table1Abbreviationsrelated to Growth Analysis and their Units.

| Abbreviation | Meaning | Units |
|---|---|---|
| **LAR** | Leaf Area Ratio | $cm^2\ g^{-1}$ |
| **LMR** | Leaf Mass Ratio | $g\ g^{-1}$ |
| **NAR** | Net Assimilation Rate | $g\ cm^2\ week^{-1}$ |
| **RGR** | Relative Growth Rate | $g\ g^{-1}\ week^{-1}$ |
| **RWR** | Root Weight Ratio | $g\ g^{-1}$ |
| **SLA** | Specific Leaf Area | $cm^{-2}\ g^{-1}$ |
| **TDW** | Total Dry Weight | mg / g |

## Table of Contents

List of tables

List of figures

## Introduction

Any biological species which becomes established outside its native habitat and aggressively outcompetes the native species is called an invasive alien species (Tiwari, 2005). The Global invasive species programme (GISP) has defined invasive species as exotic species which are introduced and established beyond their native habitat causing significant harm to the local environment, economic system and human health (Lowe et al., 2000). Invasive alien plant species have vigorous growth and capable to form monocultures and thereby outcompete the native species (Mack et al., 2000). The invasive alien species also have serious environmental and socio-economic problems (Zheng et al., 2009). It has been reported that invasive aliens are the second largest threat to bio-diversity loss next to the habitat destruction (Randall, 1996). Because of these reasons, invasive species and their invasiveness has become a common field of research among ecologists during the last few decades. The spread of invasive alien species have become a global issue as a result, research activities have been increasing in field of the ecology. To identify the underlying causes and mechanisms of invasion success is the major goal of the researchers. The findings from the investigation can be helpful to predict and control the outbreak of invasive species in particular ecosystems.

*Mikania micrantha* (hereafter *Mikania*) is a notorious perennial vine belonging to the family Asteraceae, originating from tropical central and South America (Holm et al., 1977). Out of its native range, *Mikania* has been widespread as an invasive species in subtropical and tropical Asian countries like China, India, Bangladesh, Sri Lanka, Nepal, Malaysia, Thailand, Indonesia, Vietnam, Singapore and Pacific Islands (Yang, 2005). This species also has been reported in Australia; in North Queensland, *Mikania* has been listed as a class 1 weed by Land protection (Pest and stock Route management) Act 2002 (QDPI&F, 2007). Recently, the United States department of Agriculture (USDA) has declared *Mikania* as a serious agricultural and environmental weed (Weaver Jr and Dixon, 2010). In India *Mikania* has been reported as a major problematic weed in tea gardens particularly in the north-east and south-west states (Puzari, 2010). The state level environmental protection administration of China has listed *Mikania* among the top invasive species (Zhang et al., 2004). The world conservation union (IUCN) has recognized *Mikania* as a major invasive alien species of Nepal and categorized it as high risk posed IAS (Tiwari, 2005). *Mikania* invasion is a serious problem in Chitwan national park (CNP) and Koshi tappu wildlife reserve in Nepal causing significant damage to native flora as well as habitat problems and grazing problems to wildlife (Siwakoti, 2008). The rapid expansions of *Mikania* throughout the community forest have severely affected the livelihood of local people in national park territory (Sapkota, 2009). In addition to ecological damage (Yang, 2005), *Mikania* has been widely reported to damage agro-forestry systems such as tea plantations, sugarcane, banana, rubber, teak, oil palm, coconut and *Shorea robusta* plantations (Yang, 2005). This species has been identified as one of the worst 10 weeds and one of the worse 100 invasive alien species(Lowe et al., 2000)

Ecologists are unanimously agreed on the fact that success of plant invasion in an ecological community is driven by life history traits (morphology, reproductive and physiology) of the invader and ecological factors of the invaded ecosystem (Williamson, 1997). There is no single or unified explanation for invasive alien species establishment because the invasion success of particular species is a complex interaction between the invader and introduced community (Radford and Cousens, 2000). There have been numerous hypotheses and explanations proposed by invasion ecologists to explain the success of invasive species establishment outside their native range. The fluctuating resource hypothesis is the most convincing one and is able to address the arguments (Davis et al., 2000). Nutrient rich habitats enhance the competence in the favour of invasive species which can efficiently utilize the soil nutrient for faster growth (Maron and Connors, 1996). Evidently, addition of fertilizer in California serpentine grassland had substantially increased the dominance of invasive species displacing the native forbs in a long run experiment (Huenneke et al., 1990). Another experimental study on plant invisibility in limestone grasslands of Great Britain observed the dominant presence of invasive species in nutrient-rich sites accompanied by intense disturbance events (Burke and Grime, 1996). Furthermore, long term nutrient supply experiment on Minnesota grassland shifted natively dominated grassland to non-native species of grasses (Wedin and Tilman, 1996). Many studies have been done across closely related invasive and non-invasive congeners to compare the traits associated with invasive characteristics. Based on these studies it is postulated that exotic invasive plants have higher resource capture and utilization capacity as compared to non-invasive species. The higher resource capture and efficient utilization of resources enable the invasive species to better utilize available sunlight and nutrient resources (Shen et al., 2011). Resource rich habitats with disturbance such as agricultural activities are more prone to invasion success (Holm et al., 1977). A study on the invasive species *Lantana camara* shows that soil fertilization alone had only small effect on invasion success but the increased availability of other resources such as light and water with disturbance factors play a combined role in invasion success (Duggin and Gentle, 1998). According to the previous researchers in this field of plant invasion ecology, high nutrient availability facilitates the invasion success in different vegetation communities (Lake and Leishman, 2004, Bashkin et al., 2003). Hence it is reasonable to study the plant invasiveness in relation to resource availability because invasiveness of plant species is associated with resource availability in introduced habitat(Schumacher et al., 2009).

In addition to soil nutrient resources irradiance is another vital resource for growth, development and reproduction of invasive plants. Recent publications on invasion ecology revealed that exotic invaders have a greater capacity to efficiently utilize high light than non-invasive species (Feng et al., 2007). These invaders employ maximum utilization of light energy by producing more foliage organs essential for growth and development(Shen et al., 2011). Invasive plant species can efficiently capture and utilize light resources to perform higher photosynthetic rate (Pattison et al., 1998). This strategy of higher opportunistic resource capture and efficient utilization of resources for growth and development is an important trait associated with plant invasiveness (Burns, 2006). The higher degree of physiological performance i.e. high photosynthetic rate in invasive species is associated with underlying mechanisms of invasion success (Durand and Goldstein, 2001). A comparative study on invasive *Eupatorium adenophorum* with native congeners under different level of irradiance revealed that a higher level of irradiance results in superior relative growth rate (hereafter RGR) performance than native species, which is one of the major trait for invasiveness (Zheng et al., 2009). The resource capture related traits, like specific leaf area (hereafter SLA), net assimilation rate (here after NAR) and Photosynthetic rate were higher in the invasive species *Ageratina adenophora* and *Chromolaena odorata* than non-invasive species *Gynura sp*. when grown under different levels of irradiances ( (Feng et al., 2007). The net photosynthetic efficiency was more than 1.5 fold higher in invasive species of Rosaceae than non-invasive species under higher level of irradiance (McDowell, 2002). A physiological study under different levels of irradiance and water conditions found that *Mikania* favoured high light with full soil watered condition resulting peak net photosynthetic rate (Zhang and Wen, 2009). Chlorophyll analysis of *Mikania* revealed that total leaf chlorophyll content Chl (a+b) and chlorophyll a:b ratio decreased with the decrease in light intensities (Zhang et al., 2009).

Out of the many traits studied, the high RGR trait is a strong feature of invasive plants associated with invasive characteristics in resource-rich environment (Eva Grotkopp et al., 2002) and (Grotkopp and Rejmánek, 2007). Still, these individual plant traits are not enough to predict plant invasion, hence causes lying behind the plant invasiveness are inconclusive (Mack et al., 2000). The exposition of traits and relative performance of invasive species depend on the growing conditions (light, water and nutrient) and disturbance factor (Daehler, 2003). A study on twenty-nine invasive pine species found that superior RGR performance was strongly correlated with their invasive nature in disturbed and resourceful environments (Eva Grotkopp et al., 2002). The comparative study on invasive and non-invasive species of *Tradescantia* (Conmelinaceae) demonstrated that invasive species had higher RGR than non invasive congener under nutrient rich conditions but the RGR performance of these congeners did not differ significantly in nutrient poor condition (Burns, 2004). The RGR trait of invasive species was associated with invasiveness when compared with invasive and less-invasive species grown in California (Grotkopp and Rejmánek, 2007). This result remained consistent when the RGR performance was measured with native and invasive forbs seedlings (James and Drenovsky, 2007). The higher degree of RGR leads to rapid occupation of a large space (Grime and Hunt, 1975) and captures more resources and reduces the duration of lifespan. This feature of exotic species make them successful invader in an introduced habitat.

Most of the previous researchers have focused their work in evaluating the RGR trait difference between native and invasive species in relation to resource availability and disturbances, but investigation of underlying causes behind particular species in relation to varying resources is scarce. Based on several studies and reviews, there is a general assumption that the morphological trait SLA has a greater effect on the RGR than the NAR. Broad correlative studies suggest that SLA is strongly correlated with RGR because SLA enables the plant to be exposed to sun light and assimilate $CO_2$ from the environment(Lambers et al., 2008). It has been reported that RGR and their components SLA and NAR vary according to environmental conditions (Shipley, 2002). A study on twenty-four herbaceous species from nutrient rich habitats demonstrated that higher RGR performance was achieved as a consequence of LAR and SLA trait among these species (Poorter and Remkes, 1990). A growth analysis study of twenty nine species of *Pinus* found that NAR, LWR and SLA traits were the variables to differentiate the RGR of invasive and non-invasive species of pines, but the contribution of SLA was more significant than other variables (Eva Grotkopp et al., 2002). The environmental conditions influence the morphological and physiological traits to achieve RGR differences (Villar et al., 2005). The growth performance of fast growing plants in high light and low light environments is driven by the NAR trait and LAR trait respectively (Poorter, 1999). Again, the interspecies variations in RGR were more associated with NAR in high photon flux and less associated with SLA and in contrast to this variation of RGR was contributed by SLA in low irradiance supply (Shipley, 2002). Therefore, contribution of SLA and NAR traits vary according to irradiance received by the plant (Villar et al., 2005). The higher degree of SLA trait was the major contributor to superior RGR performance of invasive species when compared with the less-invasive one (Grotkopp and Rejmánek, 2007). A meta-analysis study made general assumption that NAR trait correlated with RGR variation but under lower irradiance SLA trait contributes significant role in RGR variation (Shipley, 2006). A more recent comparative study on six native and six invasive forbs from nutrient poor habitats documented that higher RGR of invasive species was mainly contributed by higher SLA and root allocation traits (James and Drenovsky, 2007). In contrast to above studies lower SLA were measured in invasive than non-invasive species of Rosaceae (McDowell, 2002). The study of *Mikania* under manipulated light and nutrient supply helps us to understand resource related invasion success and suit of life history traits associated with environments.

In this study I have examined the biomass performance, physiological performance, RGR performance, morphological trait difference and chlorophyll content of *Mikania* under contrasting light and nutrient supplies. The general hypotheses of this study were that *Mikania* attain maximum biomass, growth and physiological performance in resource-rich environment i.e. full sunlight and nutrient added soil. Another objective of this study was to identify the major trait associated with RGR performance. The better knowledge on morphology, physiology and growth performance of *Mikania* with response to varying levels of light and nutrient can be important to understand the invasive character associated with this species.

**Growth analysis**

Relative growth rate is an increase in plant biomass per unit of mass present per unit time. RGR is an important life-history trait determined by physiological, morphological and biomass allocation components. Plant growth analysis factored RGR in to two components Leaf Area Ratio (LAR) and Net Assimilation Rate (NAR). RGR = LAR* NAR. Since LAR is the product of Specific Leaf Area (SLA) and Leaf Weight Ratio (LWR) which is the amount of leaf area per unit total plant biomass. LAR = SLA*LWR. Net Assimilation Rate (NAR) is the net result of carbon assimilation from photosynthesis including carbon losses from respiration, volatilization. The above equation makes clear that morphological (SLA) and physiological (NAR) traits and allocation patterns (LWR) make difference in RGR variation. The abbreviations and their units are mentioned in Table 1.

**Species description**

*Mikania* H. B. K. is a fast growing exotic weed, commonly known as mile-a- minute weed (English) and called by different name i.e Lahare Banmara, Bire Lahara, Tite Lahara (Nepalese) etc. In its native area *Mikania* is a common plant, found in open lands, on the forest boundaries and damp habitats like river banks, streams and lake margins (Maja and Kuo, 2008). Outside its native rage *Mikania* grows dominantly in wide range of habitats such as agriculture lands, fallow lands, wetlands, forests and forest edges etc. where fertility, humidity and moisture are high. *Mikania* has vigorous vegetative growth from nodes and each individual sexually produces over 40,000 wind dispersible seeds every year (Kuo et al., 2002). *Mikania* is a problematic weed because of its vigorous growth, proliferation and smothering neighbouring plants (Holm et al., 1977). Seed germination favours open and disturbed habitats (Kuo, 2003). It forms dense mat on the open ground and climbs up to 15 metre in height on the supporting canopy of trees, smothering light for photosynthesis, growth and developments (Zhang et al., 2004). The climbing habit of *Mikania* favours to growth densely over shrubs and trees. Generally flowering and fruiting season is from November to February. The flowers of the *Mikania* vine are white to greenish white, clustered on the lateral and apical part of stem. Mature seeds are black coloured, 1.5- 2 mm. long and are tufted with small and white hairs.

*Mikania* was first reported in the Ilam district of eastern Nepal in 1963 by a Japanese team (Tiwari, 2005). It is believed that *Mikania* was introduced to Nepal via North-east India trough tea saplings. The diverse varieties of bioclimatic regions within a small geographical area favour the introduction and establishment of invasive species in Nepal (Siwakoti, 2008)

**Seed Collection site**

Chitwan National Park (CNP) is located between $27^{0}16^{I}56^{II}$ N to $27^{0}42^{I}13^{II}$ latitude and$85^{0}50^{I}23^{II}$E to $84^{0}46^{I}25^{II}$ longitude in the sub-tropical inner terai lowland of central Nepal, covering an area of 932 $km^2$. This park includes unique and diverse ecosystems having significant value to the world from bio-diversity perspective. Due to its ecological feature and rich bio-diversity status this site is enlisted in United Nations Educational, Scientific and Cultural Organization (UNESCO) world heritage sites. Chitwan National Park (CNP) is habitat of more than 700 species of wildlife including about 50 mammalian species and endangered species like the one horned Rhinoceros, Royal Bengal tiger, Asiatic Elephant, Gaur and sloth-beer, Giant Hornbill, Bengal florican, lesser florican, Gharial crocodile and Mughal Crocodile. The floral diversity consist of more than 500 plant species comprising 3 species of Gymnosperms, 13 species of pteridophytes, 415 species of dicotyledons, 137 species of monocotyledons and 16 species of orchids. About 70% of the national park is Sal forest (*Shorea robusta*) and approximately 20% area is grasslands and flood plains (DNPWC – Annual Report 2009).

**Fig. 1** *Mikania* invasion in Chitwan national park

The *Mikania* seeds were collected from Chitwan National Park, Nepal in first week of February- 2012. Mature inflorescences were hand plucked from *Mikania* natural population of park territories. The floral bunches were sun dried for two days. About one thousand mature and healthy seeds were handpicked with the help of small forceps and then hand packed in polythene sachets.


**Fig. 2** Map of Nepal with Chitwan National Park. Photo source (Waterman, 2010)

**Greenhouse Experiment**

First of all the germination trial experiment was conducted at departmental laboratory INA for one week (21- 28 February, 2012). The Laboratory temperature was maintained at $20^0$C and light duration set-up for eight hours during germination trial period. After a successful germination trial, *Mikania* seeds were sown in rectangular plastic tray (58cm×31cm×7cm) containing sand. These sown seeds were watered every alternate day for one week (2-8 March, 2012) period. After completion of the germination period, seedlings were transplanted to a greenhouse (9 March 2012) laboratory. Greenhouse was setup $18^0$c day temperature and $15^0$c night temperature. About five hundred healthy seedlings were randomly selected and transplanted in cube shaped small pots (4cm×4cm×7cm) containing peat. These growing seedlings were watered three times a week. The first harvest was carried out on the 21st march, 2012 twenty five days after the seedlings were germinated. After first harvest, the seedlings were randomly assigned in to shade and light treatments. Shading arrangements were set-up by hanging an aluminium net over the potted plants allowing only 25% sunlight on the shading treatment. On the other side, light treatment had been set-up allowing direct sunlight in to the greenhouse chamber. After second harvest the light grown and shades plants were transplanted to the plastic pot sized 8cm×8cm×8cm. These light and shading treatments were divided into two replicates of nutrient-less and nutrient rich sub-groups after the fifth harvest. Commercial peat containing 86% sphagnum peat, 10% sand and 4% clay containing the macronutrient such as nitrogen, potassium and phosphorus were 850 mg, 170 mg and 35 mg per litre respectively was used in pot plantation. Substral vita plus brand of liquid nutrient solution containing 3.3% $NO_3$, $NH_4$, 1.3% P, 5% K, was used for nutrient supply. About seven ml. of nutrient solution was dissolved in one litre of tap water and supplied to potted *Mikania* seedlings every week. Altogether, thirteen harvests have been performed, in regular intervals of one week. Healthy seedlings were randomly selected from different treatments and gently washed in tap water to remove sand and peat from individual samples. These seedlings were labelled with sample numbers and respective treatment types then carried to the laboratory for further measurements.

Table 2 Experimental design followed for the study period. Tabulated numbers represent the number of plants harvested in each harvest event.

| Experimental set-up | Harvests | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Light&low nutrient | 20 | 20 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 5 | 5 | 5 |
| Shade&Low nutrient | | 20 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 5 | 5 | 5 |
| Light&high nutrient | | | | | | 10 | 10 | 10 | 10 | 10 | 5 | 5 | 5 |
| Shade&high nutrient | | | | | | 10 | 10 | 10 | 10 | 10 | 5 | 5 | 5 |
| Chlorophyll extraction and quantification | | | | | | | | | 20 | | 40 | | 40 |
| Photosynthesis measurement | | | | | | | | | 3 | | 3 | | |
| Potting | | | × | | | | | × | | | | | |

**Leaf Area and Biomass Measurements**

All the leaf blades of each individual seedling were cut with scissors to measure the leaf area. Total leaf area of each individual seedling was measured by an area meter LI- 3100 (LI-COR, Lincoln USA). Leaf, stem and root parts were separately labelled with sample ID. After area measurement, samples were oven dried in drying chamber for twenty hours at $80^0$C. The dry weight of leaves, stem and root was measured separately with the help of a digital weighting machine. Ultra micro-balance Mettler Toledo model UMX-2 (Switzerland) was used for the first four harvests. Sartorius analytical balance (Germany) was employed after the fourth harvest measurement. All thirteen harvests were performed with the same procedure. These measurements were used to estimate the following plant trait in each harvest.

Total Dry Weight (TDW) of individual plant was calculated by adding the dry weight of leaves, stems and roots. Relative Growth Rate with respect to total dry weight was calculated as RGR = $(\ln W_2-\ln W_1)/(T_2-T_1)$ where $W_2$ and $W_1$ were the total dry weight measured at $T_2$ and $T_1$ time interval respectively. Leaf Area Ratio (LAR) was calculated as ratio between total leaf areas measured and total dry weight of the individual plants. Specific Leaf Area (SLA) was calculated as ratio of total leaf area to the corresponding leaf dry weight of individual plants in each harvest. Leaf Weight Ratio (LWR) was calculated as the ratio between total leaf dry weights to the total dry weight of the individual plant measured. Root Weight Ratio (RWR) was calculated as the ratio between root dry weight and total dry weight of the plant. Net Assimilation Rate (NAR), the rate of increase of dry weight per unit leaf area was calculated as $((W_2-W_1)*\ln(A_2/A_1))/(A_2-A_1)*(T_2-T_1)$ where $W_1$ and $W_2$ represent initial and final total weight of plant and $A_1$ and $A_2$ represent the initial and final leaf area measured at one week interval $T_1$ and $T_2$.

Abbreviations, variable names and units were mentioned in Table 1.

**Fig. 3** *Mikania* seedlings (21 days after germination) grown under light and shade treatments (left to right).

**Fig. 4** *Mikania* seedlings (28 days after germination) grown under light and shade treatments (left to right).

Fig 5 *Mikania* seedling grown under shade without nutrient treatment (45days after germination).

Fig 6 *Mikania* seedling grown under shade with nutrient treatment (45 days after germination).

Fig 7 *Mikania* seedling grown under light without nutrient treatment (45 days after germination).

Fig 8 *Mikania* seedling grown under light with nutrient treatment (45 days after germination).

Fig 9 *Mikania* seedlings (59 days after germination). Light with nutrient, Light without nutrient, Shade with nutrient and shade without nutrient (Left to right).

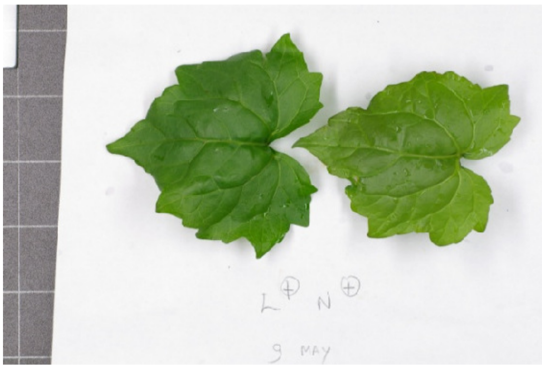



Fig 10 *Mikania* leaves (upper and lower surface) grown under light with nutrient treatment (64days after germination).

Fig 11 *Mikania* leaves (upper and lower surface) grown under light without nutrient treatment (64days after germination).


Fig 12 *Mikania* leaves (upper and lower surface) grown under shade with nutrient treatment (64days after germination).


Fig 13 *Mikania* leaves (upper and lower surface) grown under shade without nutrient treatment (64days after germination).


Fig 14 *Mikania* seedlings (after 65 days of germination). Shade without nutrient, shade with nutrient, Light without nutrient and Light with nutrient (Left to right)

**Chlolorophyll extraction**

Chlorophyll extraction was performed at 9th, 11th and 13th harvests. Fully expanded mature leaves were selected for chlorophyll sampling. A metallic cork borer with diameter 1 cm. was used to cut two circular discs of a leaf from either of each leaf mid rib. These leaf discs were put into the test tube containing 5 ml. dimethylformamide (DMF) solution. These test tubes were labelled with treatment types and stored in a refrigerator at $4^0$C for twenty four hours to extract chlorophyll content. Each test tube containing chlorophyll solution was poured with the help of pipette in to a 1.5 ml. UV cuvette (GMBH, Germany) to measure the absorbance. Every UV cuvette was subjected to chlorophyll absorbance with the help of the UV-1800-SHIMADZU-UV Spectrophotometer (Schimadzu Japan). Chlorophyll absorbance was measured at 647 nm, 664 nm and 750 nm. Chlorophyll *a* and chlorophyll *b* concentration were calculated by atomic absorption spectroscopy (Porra et al., 1989).

**Reflectance measurement**

The reflectance of light and shaded leaves was measured on the upper side at the 4th harvest. At the 5th harvest reflectance of light and shaded leaves from the upper and lower sides were measured. Light reflectance measurement was performed across the visible spectra (350-1000) nm. with the help of ocean optics SD 2000 spectrometer (ocean optics, Dunedin, Fla USA) connected to an integrating sphere (ISP – 50 – REFL ocean optics) with 400 µm fibre. Halogen light (DH 2000 ocean optics) was connected to the integrating sphere through a 600 µm fibre illuminating the sample at the sphere port. Reflectance spectra were recorded with a reflectance standard (WS – 2 ocean optics). Finally *Mikania* leaves from light and shaded treatments were placed under the integrating sphere to measure the reflectance spectra.

**Photosynthesis measurement**

Three seedlings from each treatment were randomly selected for photosynthesis measurement. Net $CO_2$ assimilation (A) with response to photosynthetic photon flux density (PPFD) was measured in a greenhouse laboratory (UMB) using a CIRAS-1 Portable infra red gas analyzer photosynthesis system (PP System, UK) with PLC 5B automatic cuvette attached to a halogen lamp. The fully expanded mature leaf was clamped inside the cuvette chamber, fixing the midrib on the middle part. $CO_2$ assimilation was measured at the following irradiance levels (PPFD) 1000, 500, 250, 100, 50 and 0 µmol photons $m^{-2}s^{-1}$, starting from highest PPFD. At the end of each experiment the halogen lamp was switched off and the cuvette was covered with cloth and dark respiration was measured. Measurements were made on sunny days between 10 a.m. to 4 p.m. The photosynthesis was measured two times at the 9th harvest and 11th harvest. Three seedling samples were selected from each treatment type to conduct the photosynthesis experiment.

**Statistical Analysis**

The normal distribution test was performed for all the calculated variables. The response of light on variables like biomass performance, physiological performance and morphological traits were analyzed by one-way ANOVA for the first five harvests. Later the effects of light and nutrient addition were analyzed by two-way ANOVA. In this statistical analysis light and nutrient were assumed as independent variables where as other variables measured were assumed as response variables. A Tukey test was used to compare the mean value between the different treatments at 95% confidence interval. All these statistical analyses were performed by using Statistical software MINITAB-16 version. The regression graph was plotted with the help of sigma plot statistical software.

## Results

### Specific leaf area (SLA)

Specific leaf area was higher in the shade grown plants than light grown plants. For instance, the average SLA of shaded plants was approximately three-times higher than the light grown plants (734 ± $cm^2g^{-1}$ vs. 257.1 ± $cm^2g^{-1}$) on the 5th harvest (Fig 15). Nutrient effect had immediately reflected on the SLA as a result nutrient added plants achieved higher SLA than nutrient less plants on the both treatments from 6th harvest. However, nutrient response was not observed after 8th harvest. In the final harvest the shaded plants without nutrient and with nutrient had highest SLA 485.8 ±15.7 $cm^2g^{-1}$ and 469.8 ± 5.8 $cm^2g^{-1}$ respectively where as the SLA from the light grown plants without nutrient and with nutrient were 231.8 ± 4.1 $cm^2g^{-1}$ and 246.3 ± 10.5 $cm^2g^{-1}$ respectively.

### Leaf area ratio (LAR)

The shaded plants had substantially greater LAR than the light grown plants. For instance, on the 5th harvest the LAR from shaded plants had more than threefold higher than the light grown plants (498 ± 22.2 $cm^2g^{-1}$ vs. 147.4 ± 4.3 $cm^2g^{-1}$). LAR had consistently increased soon after nutrient supply but this effect had not observed after 8th harvest. At last the highest LAR was measured from the shade treatment with nutrient i.e. (244.8 ± 0.01 $cm^2g^{-1}$) followed by its replicate i.e. (217.93 ± 8.56 $cm^2g^{-1}$). On the other hand LAR from the light with nutrient and without nutrient was 113.36 ± 4.79 $cm^2g^{-1}$ and 90.02 ± 2.83 $cm^2g^{-1}$ respectively which was significantly different from shade treatment. The LAR had decreased on the later stage of development (Fig 16).

**Leaf weight ratio (LWR)**

There was only marginal difference in LWR between the light and shade treatments. After addition of nutrient the LWR had substantially increased on the 7th and 8th harvests (Fig 17). There was no significant effect of nutrient supply as a consequence only marginal difference was observed. Finally the seedlings grown at shade with nutrient allocate maximum biomass to the foliage part i.e. 0.52 ± 0.01 $gg^{-1}$ followed by 0.46 ± 0.01 $gg^{-1}$ from light grown with nutrient added treatment. The LWR had decreased with plant age (Fig 17).

**Root weight ratio (RWR)**

The RWR was higher for the light grown plants than the shade grown plants and these differences were statistically significant on the 4th and 5th harvest (Table 3). The light and shaded plants without nutrient at 7th harvest had highest RWR 0.4 ± 0.016 $gg^{-1}$ and 0.31 ± 0.009$gg^{-1}$ respectively. After 7th harvest RWR of light and shade treatments without nutrient start to dipped and became lowest at the 10th harvest. The nutrient poor treatments had marginally higher RWR than the nutrient rich treatments (Fig 18).

**Net assimilation rate (NAR)**

The average NAR was substantially higher (5-fold to 6-fold) in the light grown plants than the shade grown plants (4th and 5th harvest Fig 19). The addition of nutrient had not increased the NAR except shaded plants with nutrient on the 8th harvest. The NAR of light grown plants reached at the peak level (0.07 ± 0.001 g $cm^{-2}week^{-1}$) and the shaded plants without nutrient was lowest (0.002 ± <0.054 g $cm^{-2}week^{-1}$) on the 8th harvest. Further harvest had indicated that light grown plants consistently higher NAR than shade grown plants. At last the NAR had come to the lowest level (0.001 g $cm^{-2}week^{-1}$ to 0.002 g $cm^{-2}week^{-1}$) and no significant effect of light and nutrient had observed.

**Relative growth rate (RGR)**

From the period of the 2nd harvest to the 5th harvest seedlings grown under the light treatment had measured marginally higher RGR than the shade treatment (Fig 20). Nutrient supply after the 5th harvest had indicated that shade treatment with nutrient performed maximum RGR (1.06 ± 0.18 $gg^{-1}week^{-1}$) followed by shade treatment without nutrient (1.06 ± 0.17 $gg^{-1}week^{-1}$). The RGR was not significantly different among four groups in the 7th harvest. Surprisingly the shade grown plants with nutrient achieved highest RGR i.e. 2.07 ± 0.12 $gg^{-1}week^{-1}$followed by light with nutrient treatment (1.41 ± 0.15 $gg^{-1}week^{-1}$), light without nutrient (0.84 ± 0.14 $gg^{-1}$) and shaded without nutrient (0.34 ± 0.09 $gg^{-1}week^{-1}$) respectively in the 8th harvest. At the 9th harvest light grown plants performed maximum RGR than the shade plants. Light and nutrient addition had no significant effect for the last thee harvests and RGR performance was lower than the early stages (Table 7).

**Total Dry Weight (TDW)**

The light grown seedlings had substantially higher TDW than shade grown. For instance on the 5th harvest light grown seedlings had more than twofold higher TDW (93.7 ± 8.2 mg) than shade grown seedlings (39 ± 5.8 mg). Nutrient addition had significantly positive effect on TDW for both light grown and shade grown plants. Nutrient addition had increased higher TDW under light grown plants than shade grown plants as a result of significant interaction between light and nutrient (Table 6&7). Finally, the light grown plants with nutrient had more than six-fold higher TDW (52.2 ± 2.2 g) than shaded plants without nutrient i.e. (8.1 ± 1.08 g). On the same harvest the light grown plants without nutrient and shaded plants nutrient had average TDW 22.6 ± 1.7 g and 20.4 ± 0.7 g respectively which were not significantly different (Fig 23).

**Chlorophyll content**

The total chlorophyll content per unit leaf area i.e. chlorophyll (*a* + *b*) was significantly higher in the light plants than the shade plants. Nutrient addition effect was more pronounced on the light grown plants than shade grown plants at 9th and 11th harvests. However nutrient effect was observed in shade grown plants as well in the 13th harvest. The chlorophyll *a:b* was significantly higher in the light grown seedlings than the shade grown. The nutrient addition had more effect on the light grown plants than the shade grown for chlorophyll *a:b*.

**Photosynthetic gas exchange**

The photosynthesis light response curve drawn from two experiments demonstrated that seedlings grown under light treatments had higher photosynthetic $CO_2$ uptake than the shaded plants. This study demonstrated that net photosynthetic rate of light grown plants was nearly twofold higher under full sunlight i.e. 20 µmol $CO_2m^2s^{-1}$ than shade grown plants. For light grown plants, light saturation point was above the 800 µmol $m^{-2}$ $s^{-1}$ where as the shade grown plants light saturation took approximately at the 500 µmol $m^{-2}s^{-}$1(Fig 32). Under lower irradiance level the photosynthetic $CO_2$ uptake was not significantly different between the shade and light grown plants. The nutrient addition had increased the photosynthetic rate to the higher extent which was more pronounced in the light grown plants under higher irradiance. Quantum Yield was not significantly different among the treatments (Table 9).

**Leaf reflectance**

Leaf reflectance of light grown and shade grown leaves were not different (Fig 24) on the 4th harvest. On the 5th harvest reflectance of green light was higher on the shade leaf than the light leaf (Fig 25).

In the first harvest the average TDW, SLA, LAR and LWR of the seedlings were measured as 1mg, 892.1 $cm^2$ $g^{-1}$, 455.0 $cm^2$ $g^{-1}$ and 0.53 g $g^{-1}$ respectively.

Table 3 One-way ANOVA test across two light levels

| | 2nd Harvest | | | 3rd Harvest | | | 4th Harvest | | | 5th Harvest | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Light | | | Light | | | Light | | | Light | | |
| df (1) | F-Ratio | **P-Value** | $r^2$ (adj) | F-ratio | **P-value** | $r^2$ (adj) | F-ratio | **P-value** | $r^2$ (adj) | F-ratio | **P-value** | $r^2$ (adj) |
| **TDW** | 1.38 | 0.247 | 0.96 | 1.92 | 0.183 | 4.61 | 47.15 | **<0.05** | 70.84 | 29.50 | **<0.05** | 60.00 |
| **SLA** | 65.47 | **<0.05** | 62.31 | 105.00 | **<0.05** | 84.55 | 169.51 | **<0.05** | 89.87 | 286.34 | **<0.05** | 93.76 |
| **LAR** | 44.76 | **<0.05** | 52.88 | 162.86 | **<0.05** | 89.49 | 128.21 | **<0.05** | 87.01 | 241.44 | **<0.05** | 92.68 |
| **LWR** | 11.5 | **0.002** | 21.21 | 3.09 | 0.096 | 9.90 | 1.98 | 0.177 | 4.89 | 37.95 | **<0.05** | 66.04 |
| **RGR** | 0.39 | 0.537 | 0.00 | 0.37 | 0.552 | 0.00 | 26.71 | **<0.05** | 57.51 | 1.13 | 0.301 | 0.69 |
| **NAR** | 8.80 | **0.005** | 16.67 | 13.08 | **0.002** | 38.87 | 93.30 | **<0.05** | 82.93 | 21.07 | **<0.05** | 51.36 |
| **RWR** | | | | | | | 13.62 | **0.002** | 39.92 | 66.46 | **<0.05** | 77.50 |

Table 4 Effect of light, nutrient and their interaction on different variables of *Mikania* according to two-way ANOVA test

| | 6th Harvest | | | | | | | 7th Harvest | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Light | | Nutrition | | Light × Nutrition | | | Light | | Nutrition | | Light × Nutrition | | |
| df(1) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) |
| **TDW** | 12.07 | **0.001** | 2.35 | 0.134 | 1.09 | 0.302 | 24.30 | 0.21 | 0.648 | 0.01 | 0.753 | 2.38 | 0.132 | 0.00 |
| **SLA** | 321.16 | **<0.05** | 72.45 | **<0.05** | 20.07 | **<0.05** | 91.33 | 399.61 | **<0.05** | 103.67 | **<0.05** | 19.76 | **<0.05** | 93.02 |
| **LAR** | 102.79 | **<0.05** | 31.47 | **<0.05** | 2.92 | 0.096 | 77.48 | 189.51 | **<0.05** | 169.63 | **<0.05** | 22.95 | **<0.05** | 90.67 |
| **LWR** | 1.08 | 0.306 | 0.04 | 0.848 | 4.09 | 0.051 | 5.35 | 16.63 | **<0.05** | 211.43 | **<0.05** | 4.96 | 0.032 | 85.50 |
| **RGR** | 19.82 | **<0.05** | 0.84 | 0.365 | 0.26 | 0.615 | 31.48 | 0.01 | 0.912 | 1.09 | 0.304 | 0.22 | 0.643 | 0.00 |
| **NAR** | 0.09 | 0.761 | 0.10 | 0.754 | 0.34 | 0.563 | 0.00 | 2.57 | 0.117 | 0.17 | 0.681 | 0.01 | 0.931 | 0.00 |
| **RWR** | 19.00 | **<0.05** | 24.49 | **<0.05** | 1.33 | 0.257 | 51.74 | 52.33 | **<0.05** | 323.75 | **<0.05** | 0.01 | 0.926 | 90.54 |

Table 5 Effect of light, nutrient and their interaction on different variables of *Mikania* according to two-way ANOVA test.

| 8th Harvest | | | | | | | | 9th Harvest | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Light | | Nutrition | | Light × Nutrition | | | Light | | Nutrition | | Light × Nutrition | | |
| df (1) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) |
| **TDW** | 0.00 | 0.951 | 67.42 | **<0.05** | 2.06 | 0.160 | 63.00 | 216.34 | **<0.05** | 402.92 | **<0.05** | 37.54 | **<0.05** | 94.37 |
| **SLA** | 29.55 | **<0.05** | 17.59 | **<0.05** | 0.08 | 0.773 | 53.14 | 64.23 | **<0.05** | 0.16 | 0.688 | 0.36 | 0.555 | 61.29 |
| **LAR** | 25.66 | **<0.05** | 50.59 | **<0.05** | 0.00 | 0.947 | 65.26 | 50.09 | **<0.05** | 0.29 | 0.596 | 0.00 | 0.957 | 54.85 |
| **LWR** | 0.22 | 0.642 | 106.31 | **<0.05** | 1.43 | 0.240 | 72.91 | 10.77 | **0.002** | 0.00 | 0.987 | 3.68 | 0.063 | 22.70 |
| **RGR** | 0.37 | 0.544 | 83.04 | **<0.05** | 20.85 | **<0.05** | 72.20 | 51.78 | **<0.05** | 0.32 | 0.576 | 2.40 | 0.130 | 56.90 |
| **NAR** | 13.99 | **0.001** | 6.33 | **0.016** | 11.01 | **0.002** | 42.07 | 89.79 | **<0.05** | 4.74 | **0.036** | 0.010 | 0.937 | 70.12 |
| **RWR** | 4.80 | **0.035** | 280.94 | **<0.05** | 0.53 | 0.471 | 87.90 | 3.35 | **0.008** | 209.74 | **<0.05** | 27.02 | **<0.05** | 85.87 |

Table 6 Effect of light, nutrient and their interaction on different variables of *Mikania* according to two-way ANOVA test.

| 10th Harvest | | | | | | | | 11 th Harvest | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Light | | Nutrition | | Light × Nutrition | | | Light | | Nutrition | | Light × Nutrition | | |
| df (1) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) |
| **TDW** | 238.25 | **<0.05** | 88.71 | **<0.05** | 25.73 | **<0.05** | 89.97 | 56.74 | **<0.05** | 38.56 | **<0.05** | 16.18 | **0.001** | 85.09 |
| **SLA** | 62.8 | **<0.05** | 0.01 | 0.940 | 3.31 | 0.077 | 61.81 | 462.91 | **<0.05** | 25.04 | **<0.05** | 13.86 | **0.002** | 96.33 |
| **LAR** | 45.37 | **<0.05** | 0.43 | 0.518 | 2.49 | 0.123 | 53.73 | 195.74 | **<0.05** | 0.79 | 0.386 | 0.03 | 0.856 | 91.06 |
| **LWR** | 6.15 | **0.018** | 3.00 | 0.092 | 0.01 | 0.932 | 13.64 | 0.26 | 0.615 | 0.77 | 0.394 | 0.45 | 0.511 | 0.00 |
| **RGR** | 37.31 | **<0.05** | 112.86 | **<0.05** | 18.83 | <0.05 | 80.98 | 0.18 | 0.681 | 1.96 | 0.181 | 1.33 | 0.266 | 2.39 |
| **NAR** | 78.09 | **<0.05** | 49.17 | **<0.05** | 0.37 | 0.547 | 76.17 | 9.50 | **0.007** | 3.54 | 0.078 | 2.23 | 0.155 | 39.24 |
| **RWR** | 2.49 | 0.123 | 49.91 | **<0.05** | 9.10 | 0.005 | 60.00 | 0.00 | 0.966 | 31.95 | **<0.05** | 0.13 | 0.721 | 60.49 |

Table 7 Effect of light, nutrient and their interaction on different variables of *Mikania* according to two-way ANOVA test.

| 12th Harvest | | | | | | | | 13 th Harvest | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | Light | | Nutrition | | Light × Nutrition | | | Light | | Nutrition | | Light × Nutrition | | |
| df (1) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) | F-ratio | **P-value** | F-ratio | **P-value** | F-ratio | **P-value** | $r^2$ (adj) |
| **TDW** | 138.86 | **<0.05** | 83.80 | **<0.05** | 25.31 | **<0.05** | 92.80 | 228.20 | **<0.05** | 186.99 | **<0.05** | 32.37 | **<0.05** | 95.90 |
| **SLA** | 189.56 | **<0.05** | 60.17 | **<0.05** | 16.45 | **0.001** | 93.27 | 561.59 | **<0.05** | 0.01 | 0.942 | 2.28 | 0.150 | 96.72 |
| **LAR** | 179.60 | **<0.05** | 13.57 | **0.002** | 10.65 | **0.005** | 91.36 | 492.85 | **<0.05** | 18.46 | **0.001** | 0.09 | 0.768 | 96.40 |
| **LWR** | 29.71 | **<0.05** | 22.87 | **<0.05** | 0.38 | 0.546 | 72.45 | 46.97 | **<0.05** | 68.60 | **<0.05** | 0.00 | 0.980 | 85.56 |
| **RGR** | 1.41 | 0.252 | 0.90 | 0.356 | 0.47 | 0.503 | 0.00 | 1.52 | 0.236 | 0.17 | 0.685 | 0.37 | 0.554 | 0.00 |
| **NAR** | 8.34 | **0.011** | 0.55 | 0.468 | 1.30 | 0.271 | 27.46 | 0.30 | 0.591 | 0.00 | 0.995 | 0.66 | 0.428 | 0.00 |
| **RWR** | 11.25 | **0.004** | 23.37 | **<0.05** | 0.86 | 0.368 | 63.09 | 17.06 | **0.001** | 48.42 | **<0.05** | 0.16 | 0.696 | 76.73 |

Table 8 Effect of light, nutrient and their interaction on chlorophyll *a:b* ratio and total chlorophyll content (*a+b*) of *Mikania* according to two-way ANOVA test.

| **Harvest No** | **Varables** | **Treatment types** | | | | **Light** | | **Nutrient** | | **Light × Nutrient** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Light&Low nutrient** | **Light&High nutrient** | **Shade&Low nutrient** | **Shade&High nutrient** | **F-ratio** | **P-value** | **F-ratio** | **P-value** | **F-ratio** | **P-value** |
| 9 | *Chl a:b* | 3.6 | 3.7 | 3.3 | 3.3 | 22.12 | **<0.05** | 0.93 | **0.035** | 0.84 | 0.372 |
| 9 | *Chl a+b* | 46.4 | 49.8 | 26 | 37.6 | 63.96 | **<0.05** | 13.60 | **0.002** | 4.04 | 0.062 |
| 11 | *Chl a:b* | 3.18 | 3.27 | 2.93 | 2.84 | 9.78 | **0.003** | 0.00 | 0.995 | 0.78 | 0.384 |
| 11 | *Chl a+b* | 28.32 | 34.03 | 25.89 | 28.04 | 7.16 | **0.011** | 6.24 | **0.017** | 1.28 | 0.265 |
| 13 | *Chl a:b* | 3.69 | 3.44 | 3.2 | 3.11 | 48.09 | **<0.05** | 8.74 | 0.005 | 2.01 | 0.165 |
| 13 | *Chl a+b* | 18.98 | 34.88 | 32.3 | 40.51 | 56.31 | **<0.05** | 91.02 | **<0.05** | 9.27 | **0.004** |

Table Effect of light, nutrient and their interaction on saturated rate of photosynthesis at 1000 µmol $m^{-2}s^{-1}$photons and quantum yield of $co_2$ uptake according to two-way ANOVA test.

| 9th harvest | | | | | | |
|---|---|---|---|---|---|---|
| Net $CO_2$ uptake | Light | | Nutrition | | Light × Nutrition | |
| | F-ratio | P-value | F-ratio | P-value | F-ratio | P-value |
| Saturated rate | 23.13 | **0.001** | 0.35 | 0.57 | 0.01 | 0.929 |
| Quantum yield | 3.27 | 0.11 | 0.68 | 0.44 | 0.03 | 0.873 |
| Net $CO_2$ uptake | 11th harvest | | | | | |
| Saturated rate | 19.79 | **0.002** | 1.16 | 0.313 | 6.79 | 0.031 |
| Quantum yield | 1.36 | 0.278 | 0.3 | 0.596 | 0.18 | 0.679 |

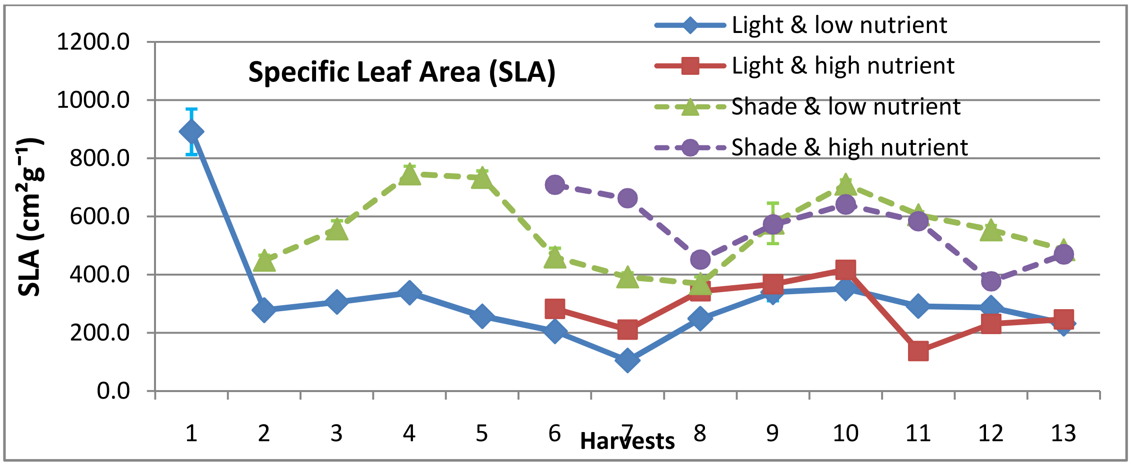


Fig. 15 Mean SLA of *Mikania* from 1st to 13th harvests. Each value represents mean of 20 samples for harvests 1 and 2, 10 samples for harvests 3 to 10 and 5 samples for harvests 11 to 13. Error bars show standard error.

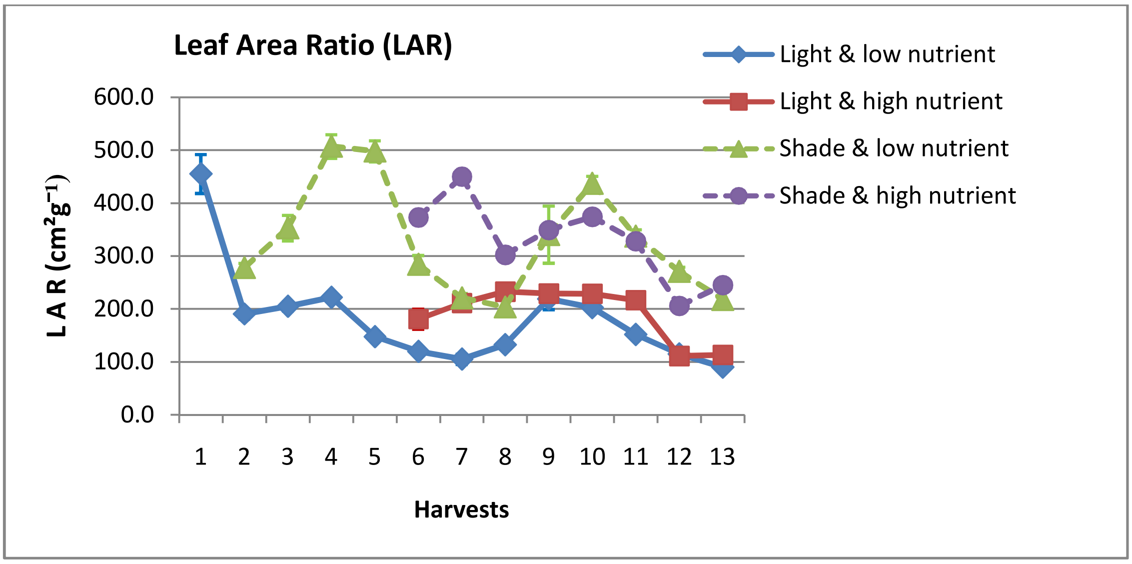


Fig. 16 Mean LAR of *Mikania* from 1st to 13th harvests. Each value represents mean of 20 samples for harvests 1 and 2, 10 samples for harvests 3 to 10 and 5 samples for harvests 11 to 13. Error bars show standard error.

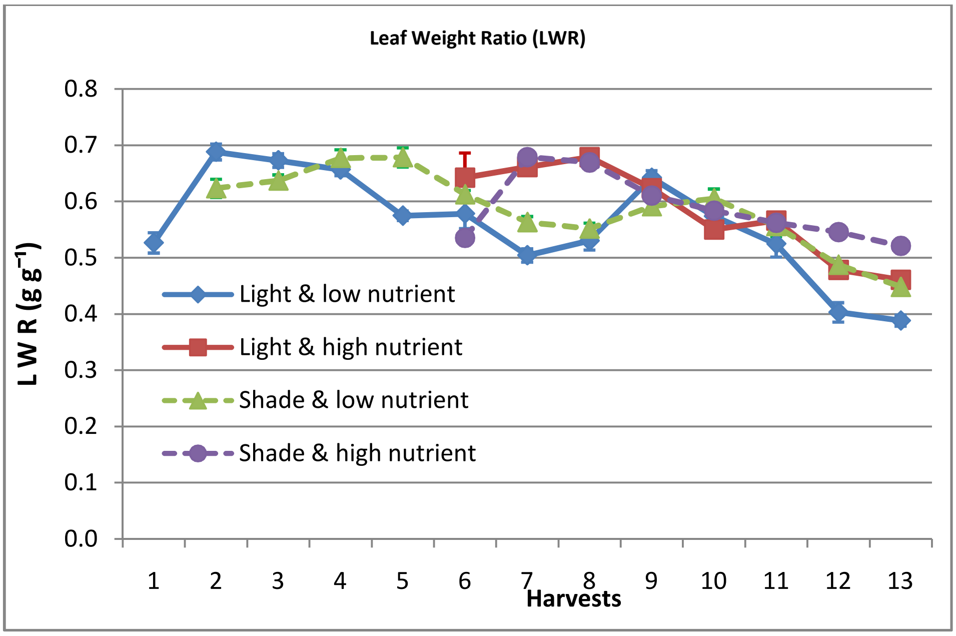


Fig. 17 Mean LWR of *Mikania* from 1st to 13th harvests. Each value represents mean of 20 samples for harvests 1 and 2, 10 samples for harvests 3 to 10 and 5 samples for harvests 11 to 13. Error bars show standard error.

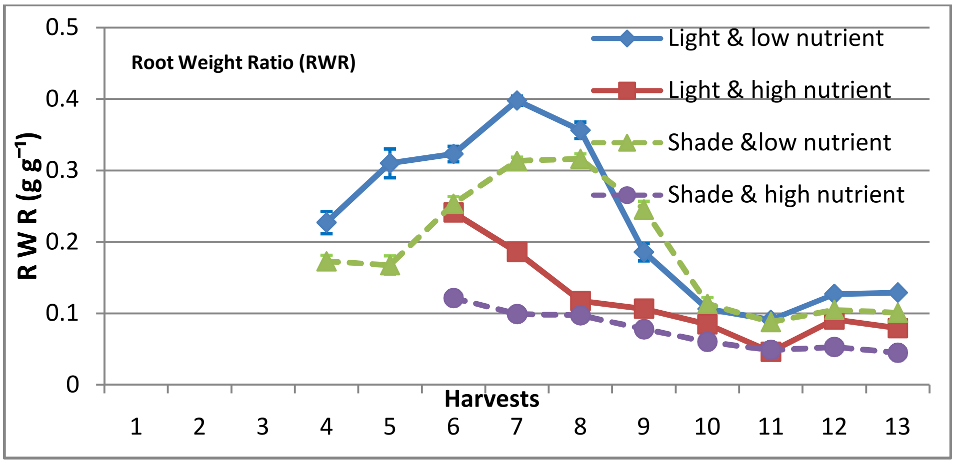


Fig. 18 Mean RWR of *Mikania* from 4th to 13th harvests. Each mean value represents the 10 samples from 4th to 10th harvest and 5 samples from 11th to 13th harvests.

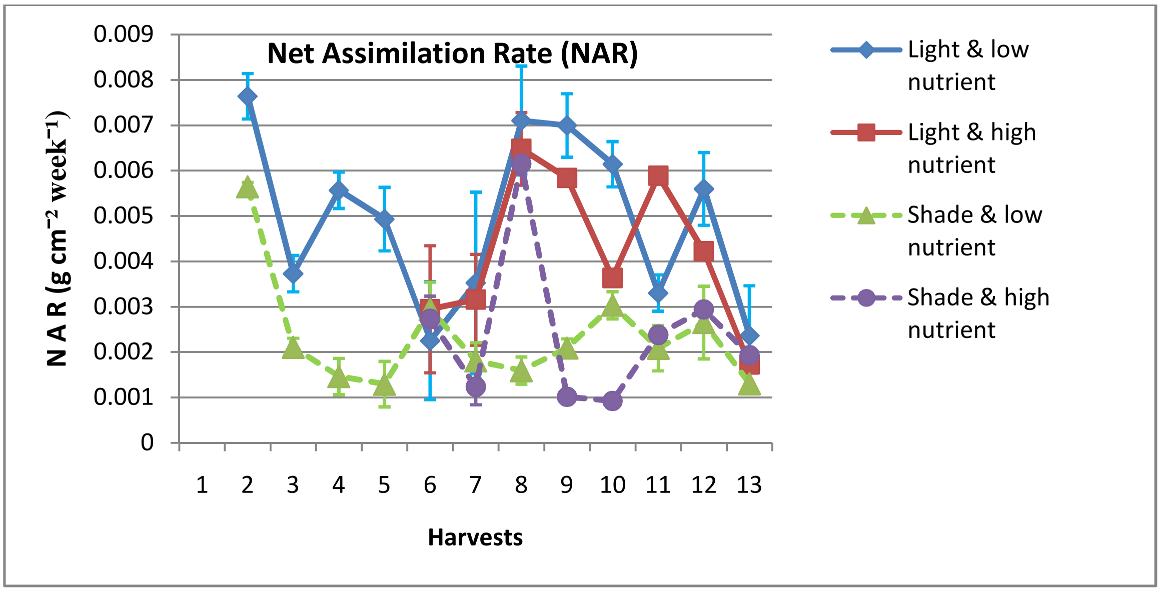


Fig.19 Mean NAR of *Mikania* from 2nd to 13th harvest. Each value represents mean of 20 samples for 2nd harvest, 10 samples for harvests 3 to 10 and 5 samples for harvests 11 to 13. Error bars show standard error.

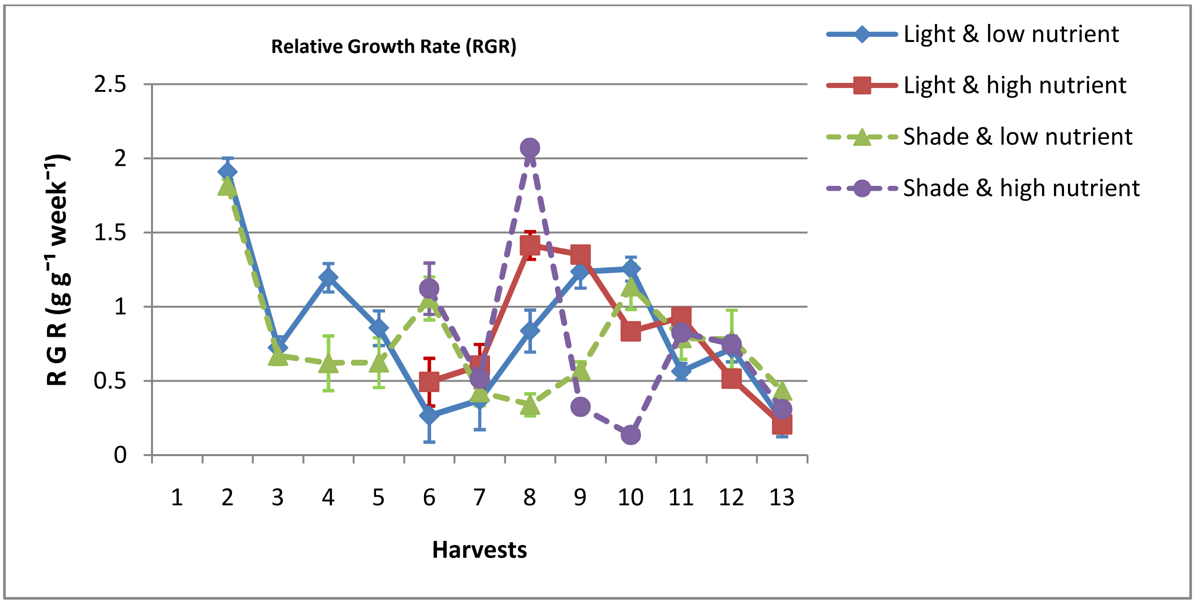


Fig. 20 Mean RGR of *Mikania* from 2nd to 13th harvests. Each value represents mean of 20 samples for 2nd harvest, 10 samples for harvests 3 to 10 and 5 samples for harvests 11 to 13. Error bars show standard error.

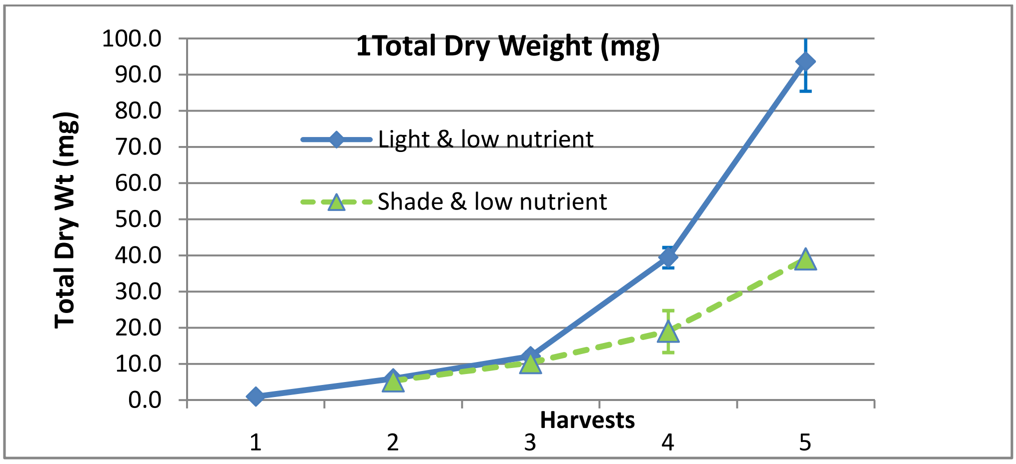


Fig. 21 Mean TDW of *Mikania* from 1st to 5th harvests. Each value represents mean of 20 samples for1st and 2nd harvest and 10 samples for harvests 3 to 5. Error bars show standard error.

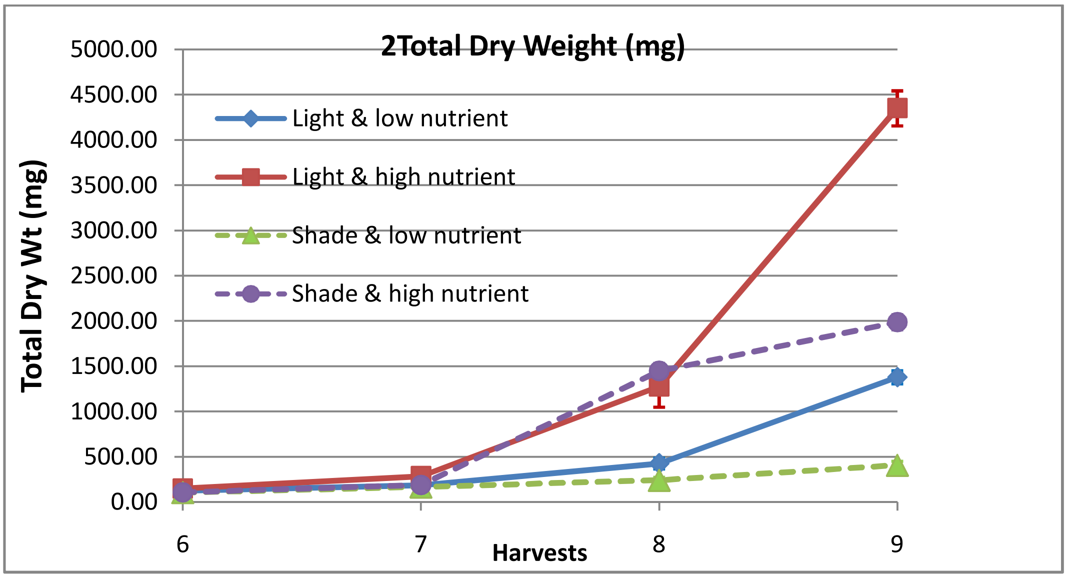


Fig. 22 Mean TDW of *Mikania* from 6th to 9th harvests. Each value represents mean of 10 samples for all harvests. Error bars show standard error

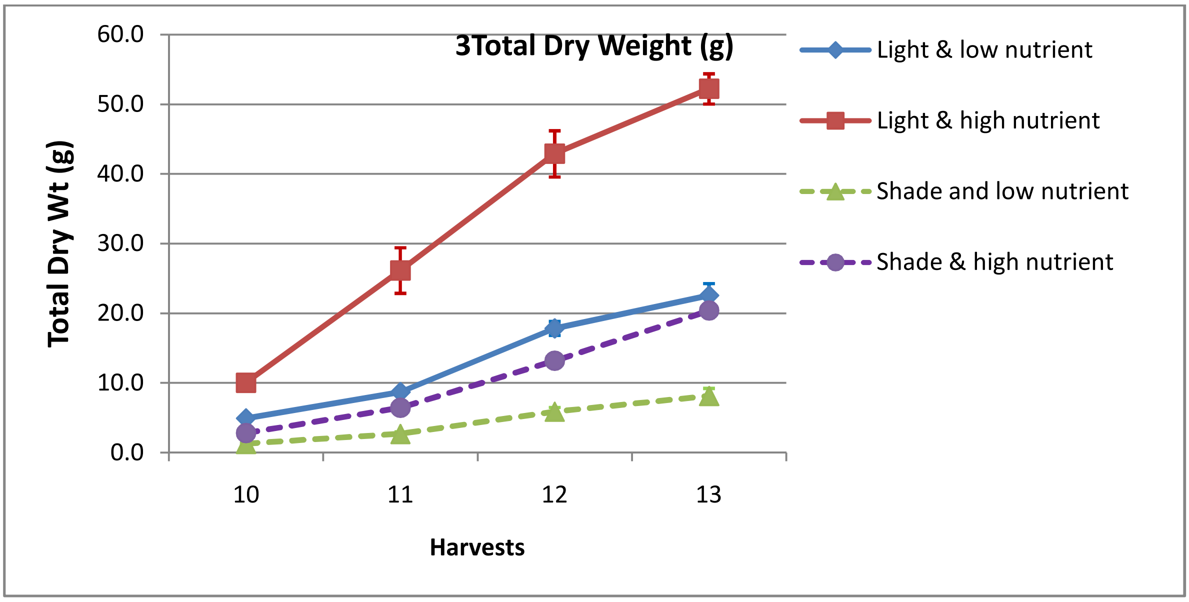


Fig. 23 Mean TDW of *Mikania* from 10th to 13th harvest. Each value represents mean of 10 samples for 10th harvest and 5 samples for 11th to 13th harvests. Error bars show standard error.

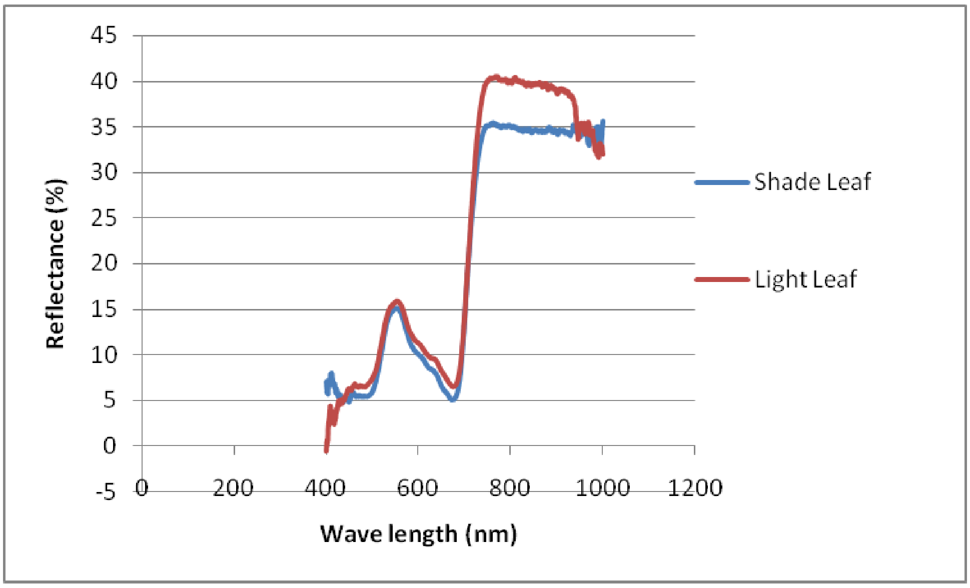


Fig. 24 Leaf reflectance of *Mikania* from upper side (4th harvest).

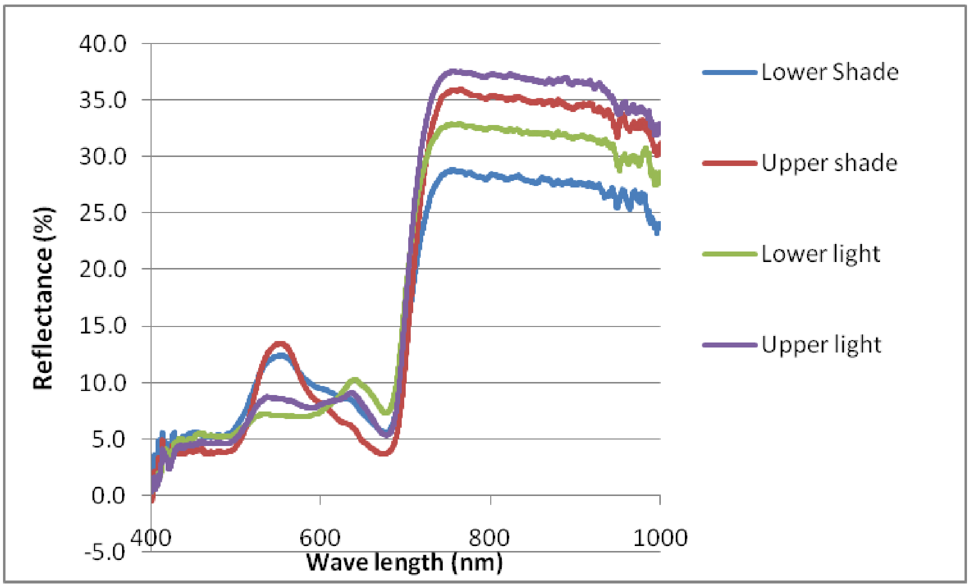


Fig. 25 Reflectance of light and shaded leaves of *Mikania* from upper and lower sides (5th harvest).

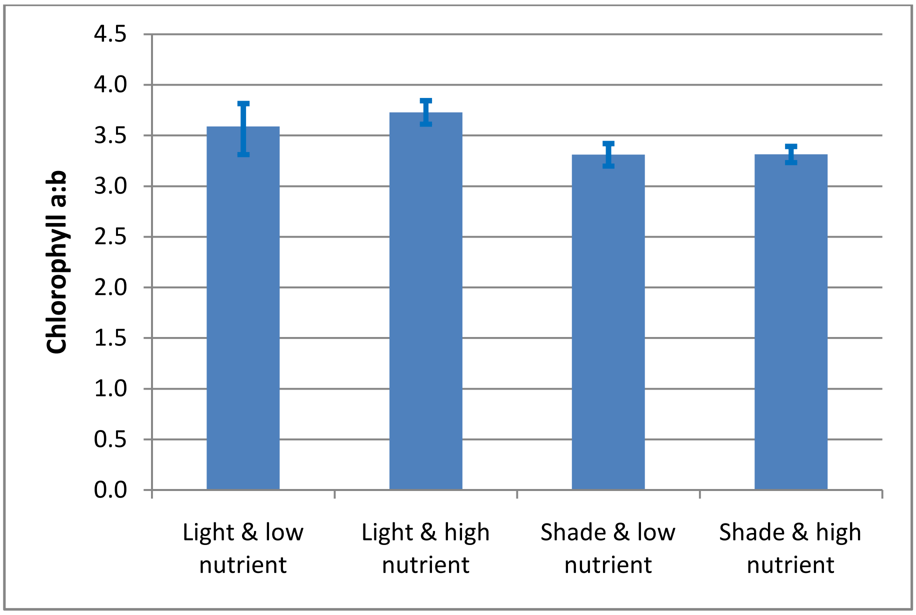


Fig. 26 Effect of light and nutrient on chlorophyll *a*:*b* ratio (9th harvest). Bars indicate standard deviation.

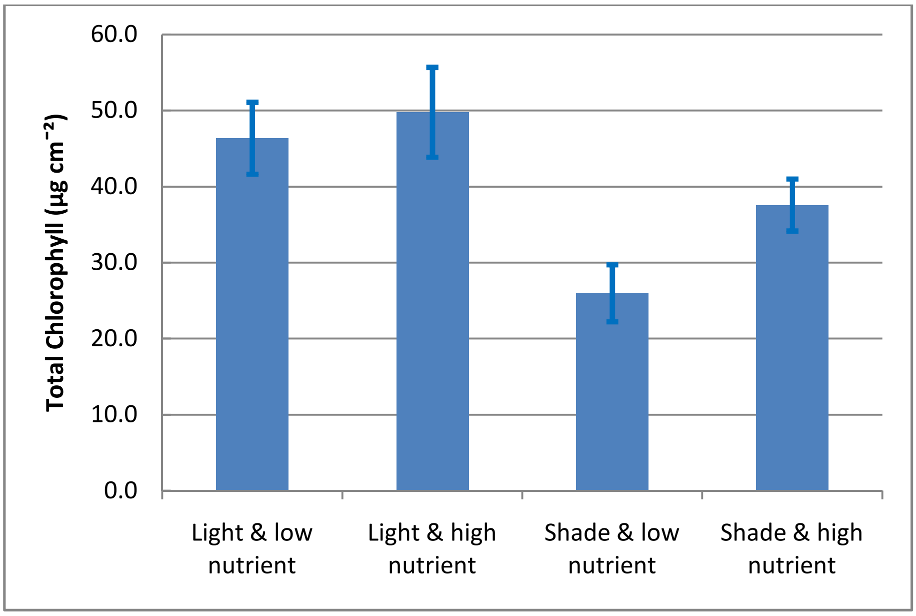


Fig. 27 Effect of light and nutrient on total chlorophyll content (9th harvest). Bars indicate standard deviation.

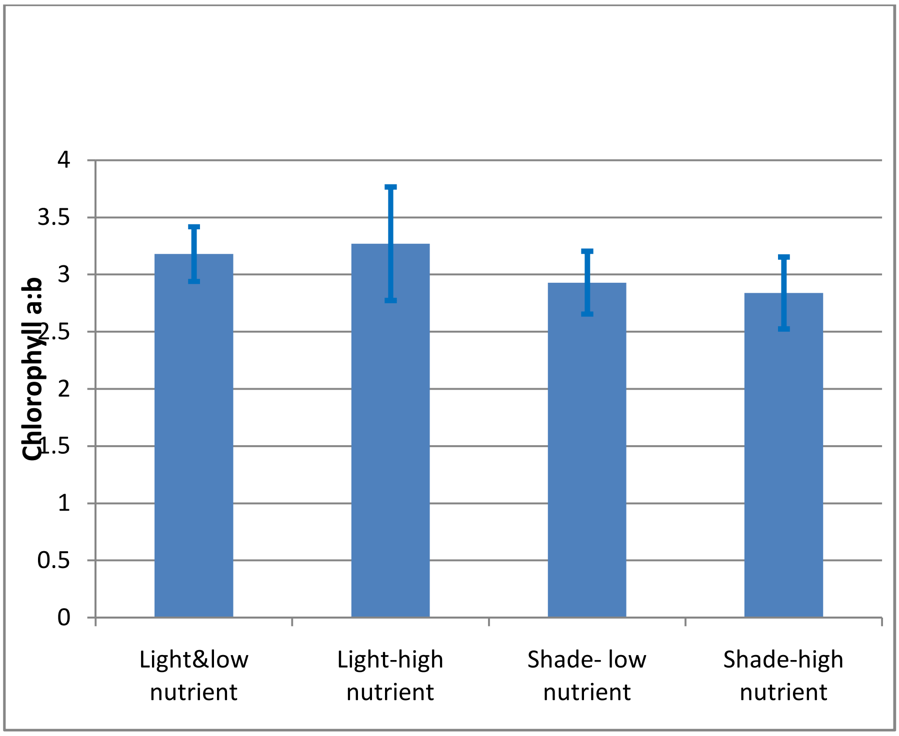


Fig. 28 Effect of light and nutrient on chlorophyll *a:b* (11th harvest). Bars indicate standard deviation.

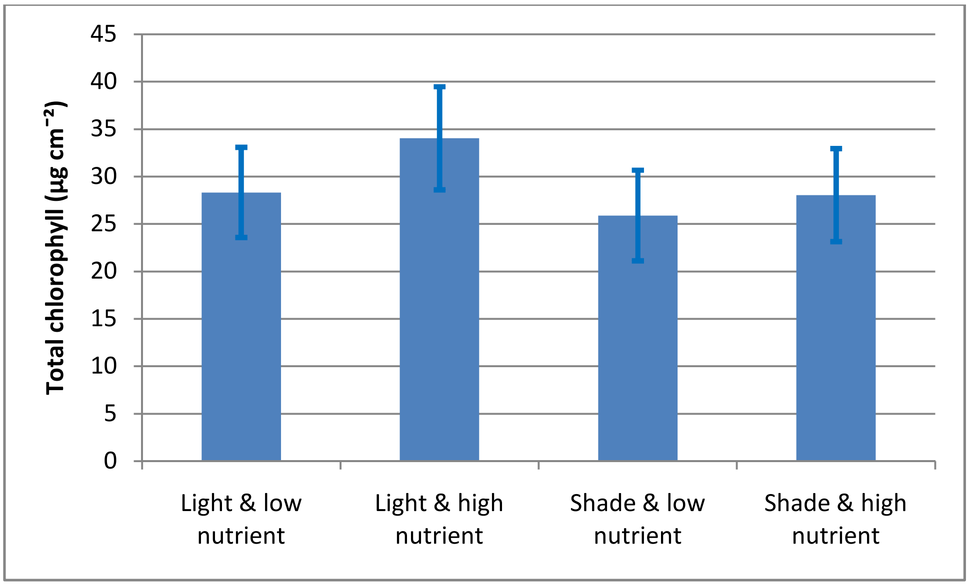


Fig. 29 Effect of light and nutrient on total chlorophyll content (11th harvest). Bars indicate standard deviation.

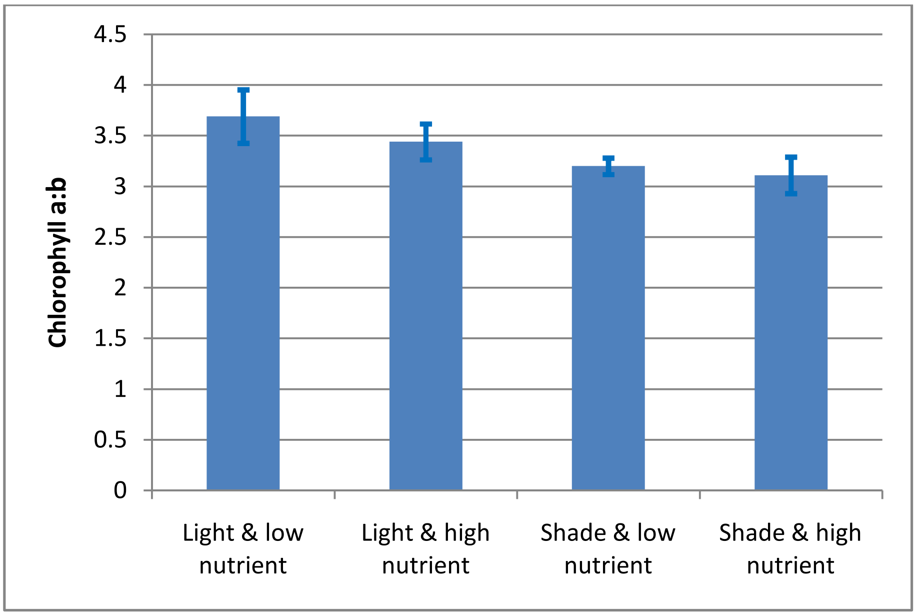


Fig.30 Effect of light and nutrient on chlorophyll *a:b* (13th harvest). Bars indicate standard deviation.

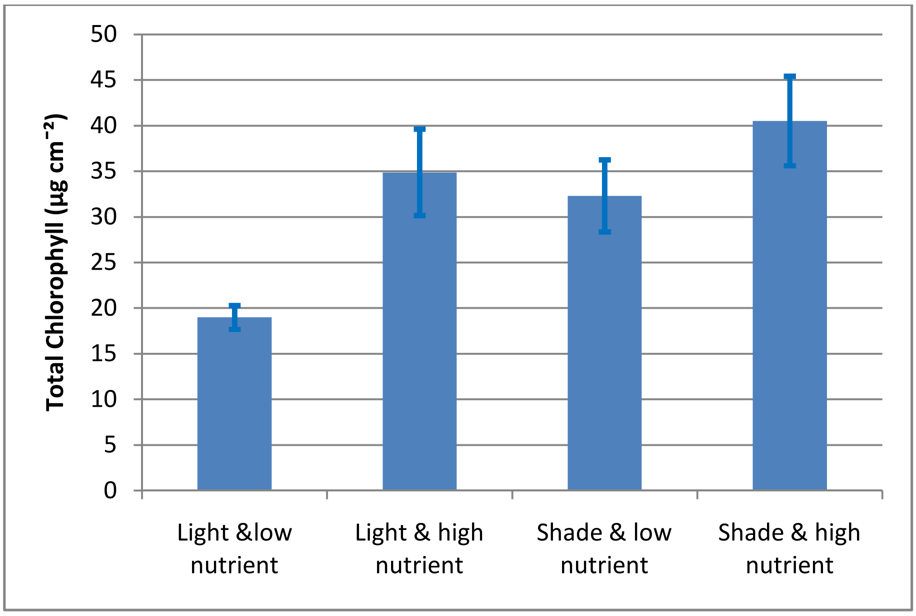


Fig. 31 Effect of light and nutrient on total chlorophyll content (13th harvest). Bars indicate standard deviation

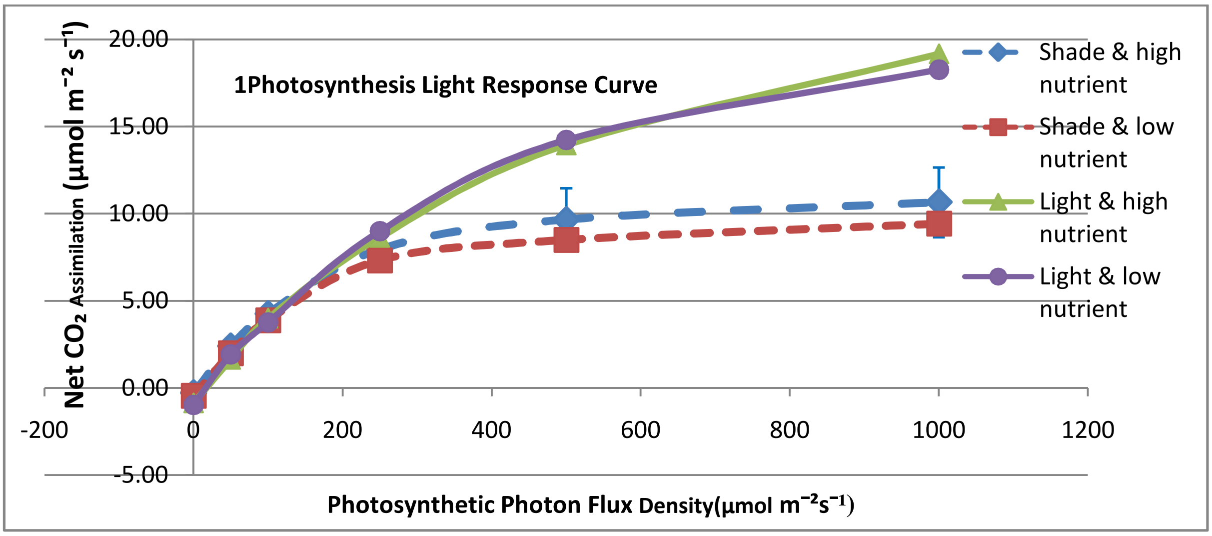


Fig. 32 Photosynthetic light response curve of *M.micrantha* seedlings grown under different treatments at 9th harvest. Bars indicate ± SE of the meas.

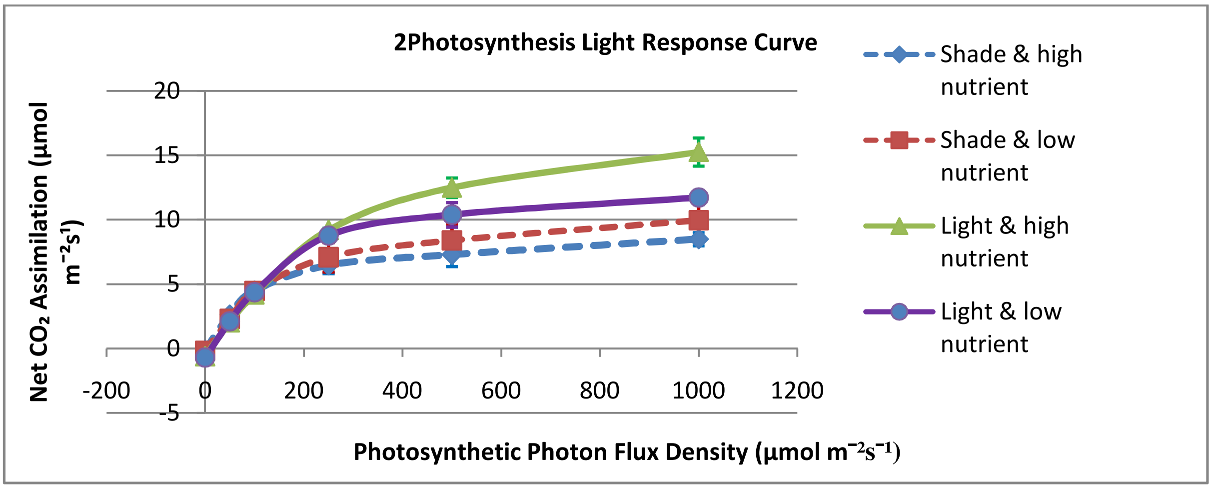


Fig. 33 Photosynthetic light response curve of *M.micrantha* seedlings grown under different treatments at 11th harvest. Bars indicate ± SE of the meas.

## Discussion

The response of light and nutrient supply on *Mikania* seedlings were studied at green house chamber. Morphology, physiology, growth and biomass performance of this species is discussed with response to resource availability comparing with previous studies of invasive species. This discussion section also addresses the traits associated with invasiveness in relation to resources availability.

### Morphological response

Morphological appearance of *Mikania* varied with response to environmental resources. SLA was found higher in the shade grown plants than the light grown plants and addition of nutrient have positive impact on the both treatments in the beginning of the experiment and this finding agrees with the previous study (Fitter and Hay, 2001). The shade-induced plastic response of *Mikania* was consistent with other invasive species *Alstonia macrophylla* (Schumacher et al., 2009). Higher SLA means thinner leaves, which are easy to produce by low construction cost in resources limited environment. Furthermore higher SLA increases the light interception surface to promote photosynthetic rate which is associated with rapid growth and development of invasive species (Lambers et al., 2008). Higher SLA is an important morphological trait associated with invasive character when they were compared with closely related native congener (Pattison et al., 1998), (Burns, 2006) and (Shen et al., 2011). However, (Feng, 2008) and (McDowell, 2002) have strong argument with this statement because invasive species like Eupatorium and *Rubus* exhibited lower SLA when grown under higher light intensities. The intra-specific comparison of SLA trait grown under contrasting light and nutrient levels don't agree that higher SLA associated with invasive character. Study on *Mikania* seedlings grown under high light and nutrient rich soil had lower SLA (thick leaves) to perform higher photosynthetic rate at lower resource investments. Besides physiological advantage lower SLA have longer lifespan to increase the lifetime carbon gain per unit leaf mass (Harrington et al., 1989). The variation in SLA between light grown and shade grown plant is an indication of light demanding species (Walters and Reich, 1999). The morphological and physiological plasticity of invasive plant to adopt different biotic and abiotic factors facilitate to successfully invade the broad range of environments (Sultan, 2003).

The response of light and nutrient on LAR trait was consistent with SLA in this study. Higher LAR of shade grown plants than the light grown plants was consistent with the invasive weed such as *Isatis tinctoria* (Monaco et al., 2005) and *Eupatorium adenophorum* (Zheng et al., 2009). The increased leaf area under low light condition was distinctly noticeable in fast growing and herbaceous species than woody species to increase carbon assimilation surface (Zdravko Baruch et al., 2000). The LWR was marginally higher in shade than light treatment and this allocation pattern increases competitive ability in light limited environment. This allocation pattern is helpful to recover carbon fixation in shaded plants. The nutrient addition effect was more pronounced on LWR than the root ratio (Smart and Barko, 1980).

The RWR was distinctly higher in plants grown under full light and nutrient less treatment. The root allocation proportion was higher from 4th to 7th harvest and reached the peak level 0.4 ± 0.016 $gg^{-1}$ and 0.31 ± 0.009 $gg^{-1}$ at light and shade treatment respectively. Plants allocate more biomass to the roots in the early stage of development for their establishment to increase the uptake of soil nutrient and water (Ledig et al., 1970). Seedlings grown at poor nutrient allocate relatively more root than nutrient enriched because plants success in nutrient poor soil depends on increased allocation to root. RWR of invasive species also facilitate higher RGR by increasing biomass allocation to above ground parts (Zheng et al., 2009).

**Physiological performance response**

Higher total chlorophyll (a+b) and chlorophyll *a*:*b* ratio in the light grown seedlings were consistent with previous study of Zhang et.al. (2009). The nutrient addition had significant effect on both variables except 13th harvest on chlorophyll *a*:*b* ratio. Higher total chlorophyll content and chlorophyll *a*:*b* ratio was advantageous for light grown plants to maximize photosynthesis under full light than shade plants.

Invasive plants get success by maximizing the photosynthetic rate (Baruch and Goldstein, 1999). *Mikania* seedlings grown under full sun light had demonstrated significantly higher photosynthetic $CO_2$ uptake under higher intensities of irradiance than the shade grown seedlings. However, apparent quantum yield under low irradiance was not significantly different between the light grown and shade grown seedlings. *Mikania* seedlings grown under full sun light opportunistically absorb more $CO_2$ for photosynthesis process under higher irradiance than the shade grown plants. This result was consistent with previous studies of invasive species as documented by (Dhillion and Anderson, 1999), (Zheng et al., 2009), (Feng et al., 2007), (Feng, 2008) and (Pattison et al., 1998). Increased absorption of higher irradiance for photosynthesis mechanism helps light favouring plants to acclimate under higher irradiance and protect photosynthetic apparatus from photo-damage (Feng, 2008). Previous studies had documented that soil nutrient play important role to promote photosynthetic rate by increasing leaf nitrogen content and leaf area (Evans, 1989). Furthermore photosynthetic rate can be increased due to interaction effect of light and nutrient (Gulmon and Chu, 1981). Indeed interaction effect of light and nutrient increased photosynthetic rates in this study as well.

**Growth performance response**

The photosynthetic performance of the seedlings clearly reflected on the NAR. As a result the seedlings grown under light treatment indeed had greater NAR than the seedlings from shade treatment. The effect of soil nutrient had followed the same trend as observed in photosynthesis measurement. Similar positive correlation between photosynthetic rate and NAR had found in intra-specific comparison with response to irradiance level (Feng et al., 2007).

The RGR was relatively higher immediately after seed germination (early stage of development) and start to decline after 10th harvest. This pattern of RGR declination agreed with study of Evans 1972. The effect of full light and nutrient addition had more pronounced on RGR in the early stage than later stage of development phase. Similar to *Mikania* other invasive species of Melastomataceae (Baruch et al., 2000) and *E.adenophorum* (Zheng et al., 2009) also exhibited higher RGR under high light supply. Pattison et. al. (1998) had studied invasive species *Schinus terebinthifolius* performed about 1.5-fold higher RGR in high light condition than shade. *Mikania* also performed similar proportion of RGR difference grown under light and shade treatments (from 2nd to 5th harvest). The variations of RGR among the different treatments were explained by variation in different traits. In this study there was only marginal difference in LWR between the light and shade treatments. As a result NAR can be major contributor to RGR under resource rich environment where as SLA as a major contributor of RGR under resource poor environment. This positive correlation between RGR and NAR (Fig 34 & 35) agreed with (Feng et al., 2007).

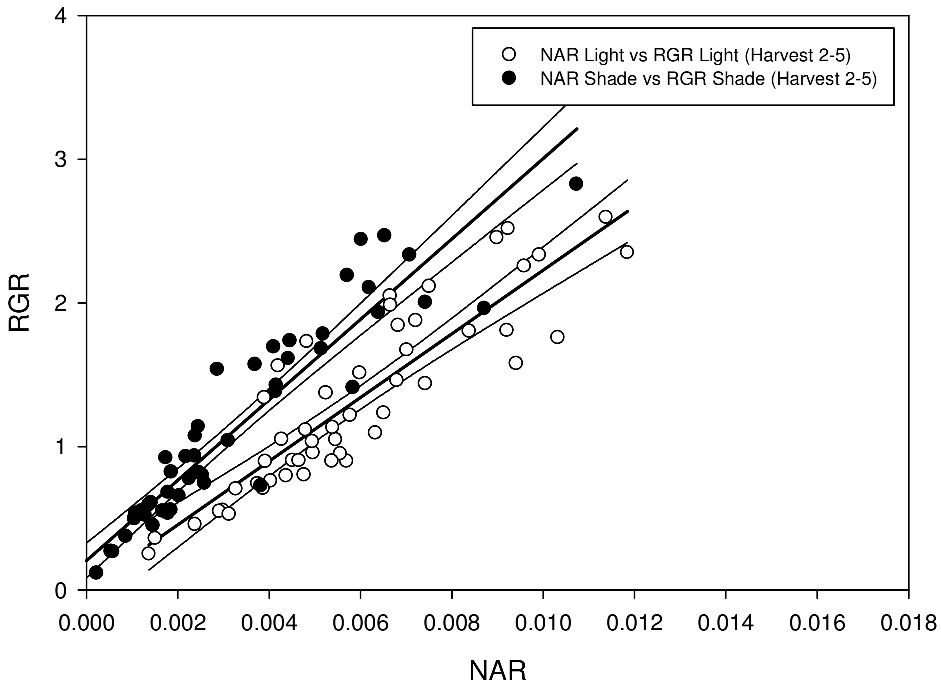


Fig. 34 Mean NAR plotted against respective mean RGR from 2nd to 5th harvest.

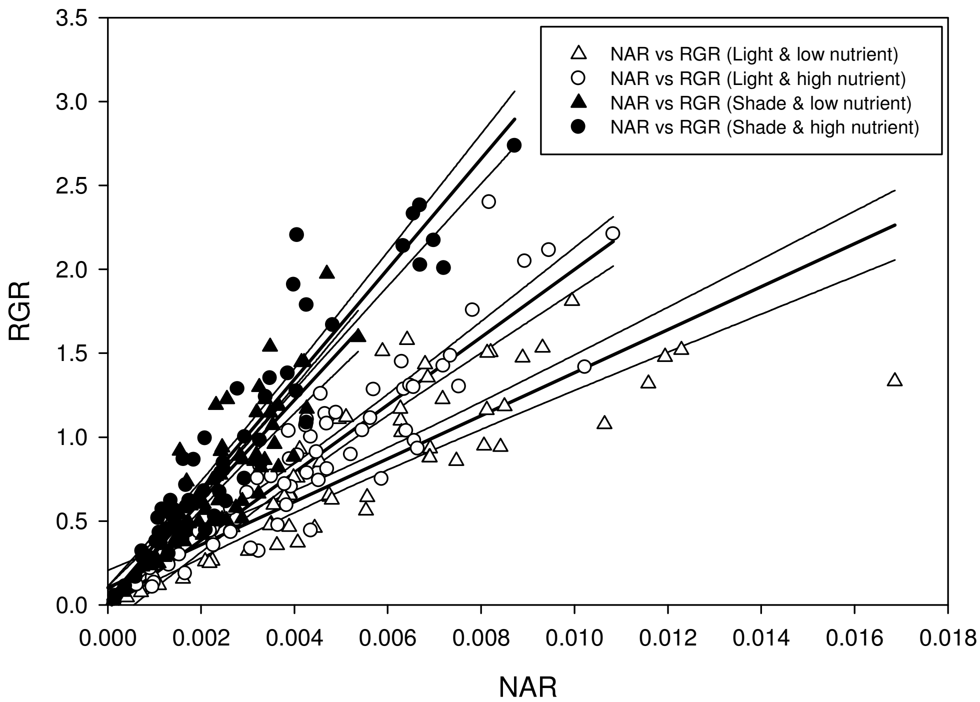


Fig. 35 Mean NAR plotted against respective mean RGR from 6th to 13th harvest.

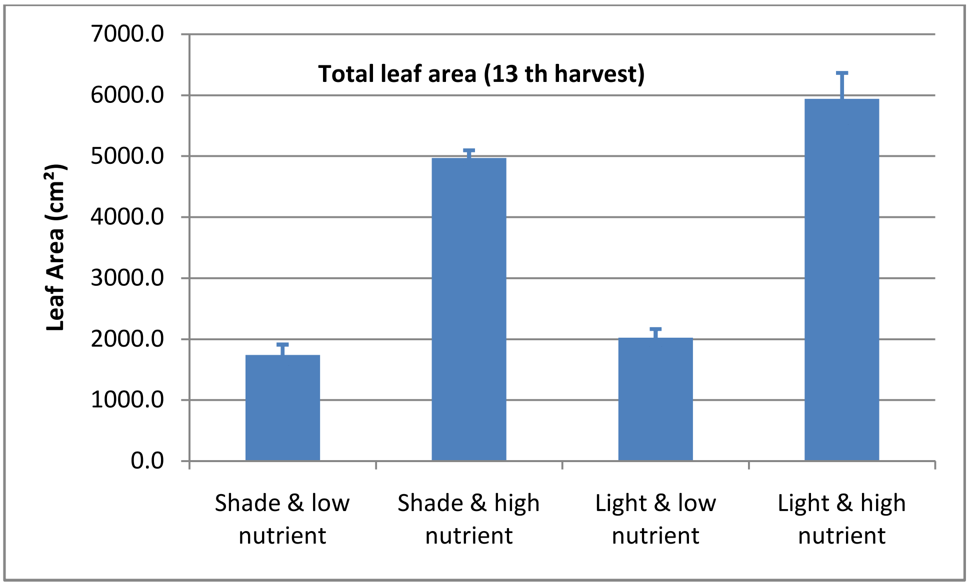


Fig.36 Total leaf area of *Mikania* grown under different treatments (13th harvest)

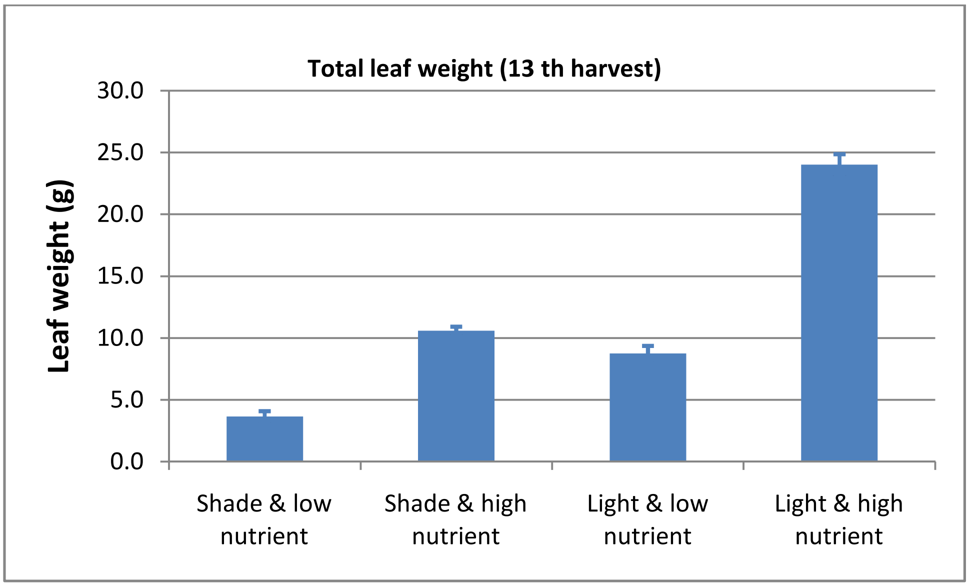


Fig.37 Total leaf weight of *Mikania* grown under different treatments (13th harvest)

**Biomass performance response**

Biomass response of a plant is an integrative result of physiological performance, morphological allocation and growth performance. Greater biomass performance of light grown plants compare to shade grown plants in this study was consistent with invasive species *E. adenophorum* (Zheng et al., 2009). From this result we can draw conclusion that *Mikania* can utilize the light resource efficiently producing more organic matter essential for growth, development and reproduction. Addition of soil nutrient to light grown plants had exhibited synergetic effect on total biomass of the plant. As a result plants grown under full light with nutrient attained maximum dry weight. Similar to *Mikania,* superior biomass and RGR performance of invasive species had studied by (Burns, 2006) and (Leishman and Thomson, 2005) due to interaction of two resources. *Mikania* adopt efficient physiological strategy and morphological allocation pattern to capture and utilize the available resources under the resource rich environment which facilitate the vigorous growth and development of the plant. In this study *Mikania* seedlings grown under full light with nutrient rich soil had performed maximum photosynthetic rate together with higher NAR, RGR and TDW. On the other side of the experiment *Mikania* seedlings grown under shade and nutrient poor soil had measured lower photosynthetic rate followed by lower NAR and TDW.

**Plant attributes and resources availability associated with *Mikania* invasion**

The plant attributes associated with invasiveness and invisibility of habitat is the central issue and challenging task in the field of invasion ecology (Van Kleunen et al., 2010). Many studies have been done in the field of invasion ecology to identify the traits associated with invasiveness and habitats infested by invasive plants. Baker (1974) had recognized that vigorous vegetative growth, vegetative propagation, production of enormous amount of seeds, non-specific pollination and germination requirements were the traits associated with plant invisibility. From the end of the 20th century many ecologists had intensified the research activities in the field of invasion ecology and started to correlate the plant attributes with their invasive character. But their studies were not well enough to convince the questions associated with invasiveness because of following reasons. Firstly, studies were extensively broad (Reichard and Hamilton, 1997, Binggeli, 1996). Secondly, there was no differentiation between introduced invasive species and non-invasive species (Melinda D. Smith and Alan K. Knapp, 2001). Thirdly, due to wide variation in environment approach (Reichard and Hamilton, 1997). Plant invasion in an introduced habitat is influenced by many factors such as propagule pressure, traits of the introduced species and environmental quality (Lonsdale, 1999). The most convincing explanation had proposed based on the growth analysis and traits exposure according to environmental conditions. The potential plant attributes associated with invasive character includes relative growth rate, specific leaf area (Eva Grotkopp et al., 2002), reproductive traits such as short life span and smaller seeds (Rejmánek and Richardson, 1996), plant acclimatisation and seed dispersal (Baker, 1974), escape from the natural enemies and herbivory damage (Schierenbeck et al., 1994) and competitors (Williamson, 1997).

Light and soil nutrient are not only fundamental resources for growth, development and reproduction of plants but these resources have prominent role in bio-invasions too (Gurevitch et al., 2008). A community is more susceptible to invasion when there is a increased amount of resources available for invasive species (Davis et al., 2000). Resources availability in a community can be increased either by declining the resource uptake from resident vegetation or increase in resource supply from external sources such as eutrophication, increased runoff, canopy removal etc (Davis et al., 2000). I have mentioned above that exposure of morphological traits and their efficiency varied with response to environmental resources such as light and soil nutrient. Invasive plant species respond to higher level of resources with higher RGR performance and under resource limited conditions respond with lower RGR (Poorter and Remkes, 1990). High RGR have fitness and competitive advantage to occupy more space and acquire resources opportunistically from resource rich environment (Matzek, 2011). Indeed *Mikania* seedlings grown under full sunlight and nutrient achieved higher RGR where as relatively low RGR was measured under shaded treatments. *Mikania* has ability to capture and utilize high level of irradiance and soil nutrient to optimize their photosynthetic capacity essential for growth and development but they can tolerate the shady environments as well (Zhang et al., 2004). As a result of higher $CO_2$ uptake NAR was increased which finally contributed for higher RGR under resource rich environment. Lower SLA (thick leaves) had performed best in photosynthetic rate in lower resource investment. In fact SLA trait is a morphological acclimatization response to efficiently capture lowlight. According to Grime and Hunt (1975) fast growers have superior growth response under plentiful resources than the slow growers and they employ more opportunistic approach to assimilate the available resources and make more resource capturing organs such as leaves and roots. *Mikania* seedlings grown under resource rich environment produce quantitatively more amount of efficient leaves which captured sun light to produce higher amount of total biomass and make them dominant species (Figure 36). *Mikania* seedlings were best adopted in resource limited environments such as shade and nutrient poor soil and expand their invasion rage whenever they receive more resources from external environment such floods, eutrophication, canopy removal and anthropogenic activities.

## Conclusion

The result from this study suggests that *Mikania* is a light and nutrient favouring invasive species. There was significant variation in morphological allocation patterns, physiological performance, growth performance and biomass performance with response to the levels of sun light and soil nutrient availability. The physiological performance and growth performance indicated that interaction of environmental resources like light and nutrient supply were very important to over perform *Mikania* seedlings under plentiful resources availability. In the resource rich environment *Mikania* seedlings employ the best utilization of available sun light and soil nutrient to maximize photosynthetic rate, net assimilation rate and relative growth rate. Higher relative growth rate under resource rich environment leads to capture more resources and occupy more space making them dominant species over the introduced habitat. Net assimilation rate is the key trait correlated with the relative growth (Figure X). Furthermore, under adverse environmental conditions morphological modifications such as higher SLA and RWR helped the *Mikania* seedlings to adapt the adverse situations. *Mikania* allocated greater proportion of root mass in nutrient poor soil and greater proportion of leaf area under shady environment to increase light interception for photosynthesis mechanism. Efficient utilization of higher intensities of sun light and soil nutrient for their physiological mechanism and deployment of efficient resource capturing organs i.e. higher amount of thick leaves with rich chlorophyll content allow *Mikania* to grow abundantly as dominant species in an introduced habitat where more light and soil nutrient are accessible.

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