Journal of Crop Science and Biotechnology (2022) 25:381–392
https://doi.org/10.1007/s12892-021-00139-9

ORIGINAL RESEARCH

The effects of pH, salinity, age of leaves, post-harvest storage duration, and psyllid infestation on nutritional qualities of giant leucaena fodder

Ahmed M. Bageel[1] · **Dulal Borthakur**[1]

Accepted: 29 December 2021 / Published online: 12 January 2022

Abstract
Giant leucaena (*Leucaena leucocephala* subsp. *glabrata*) can be managed as a profusely branched bushy plant by repeated harvest of its foliage for use as fodder. The objective of this research was to determine the effects of soil pH and salinity, age of the leaves, post-harvest storage duration, and psyllid infection on the nutritional qualities of leucaena fodder. To determine the effects of soil pH and salinity on fodder quality, giant leucaena K636 plants were grown in large pots containing soils adjusted to different pH and salinity levels. The effects of age of the leaves, post-harvest storage duration and psyllid infection on fodder quality were studied using leucaena samples collected from Waimanalo Research Station. Among five pH levels tested, pH 6.0 was found to produce the highest amounts of protein and structural fibers in the foliage. Mimosine contents were highest at pH 6 and 7 and lowest at pH 5.0. The growth of giant leucaena was retarded and the nutritional quality were adversely affected under salinity conditions. Compared to young leaves, old leaves contained 18.5% less protein, 95% less mimosine, 30% less tannin and 40% more structural fibers. Post-harvest storage duration up to 72 h, at room temperature did not seem to affect protein, tannin and structural fiber contents of the foliage; however, mimosine content was reduced by 25%. These results will help to identify ideal soil pH, age of foliage, and post-harvest storage duration for obtaining high forage yield and nutritional quality for giant leucaena.

Keywords Legume · Mimosine · Tannin · Structural fibers · Protein content

Introduction

Leucaena (*Leucaena leucocephala*) is considered an important tree legume due to its high protein content of the foliage, its ability to withstand various abiotic and biotic stresses, its adaptation to various tropical and subtropical environments, and its minimum input requirements for cultivation. Although two types of leucaena, giant leucaena (*L. leucocephala* subsp. *glabrata*) and common leucaena (*L. leucocephala* subsp. *leucocephala*) are known, only giant leucaena is cultivated for use as fodder (Bageel et al. 2020). Giant leucaena can be maintained as a dwarf shrub by repeated harvest of its foliage several times a year and it is highly responsive to favorable agricultural practices. Therefore, it can produce high green forage yields of > 100 MT/ha/year (Brewbaker et al. 1972; Elfeel and Elmagboul 2016). Common leucaena, on the other hand, produces too much seeds and less foliage and is considered an invasive species. The foliage quality of both giant and common leucaena is affected by the presence of mimosine and tannin. Mimosine is a toxic non-protein amino acid, the intake of which should not exceed 0.18 g/kg/day of total body weight of animals (Szyszka and Meulen 1984). Similarly, the amount of tannin should not exceed 50 g/kg/day of total dry matter of the foliage consumed by an animal (Tanner et al. 1995). Ideally, a good-quality leucaena foliage should contain a high amount of protein, least amount of mimosine, moderate amounts of tannin and low total structural fibers. These four parameters are affected by different stresses and factors; such as, soil pH and salinity, age of the foliage, post-harvest storage, and diseases.

✉ Dulal Borthakur
dulal@hawaii.edu

Ahmed M. Bageel
abageel@hawaii.edu

1 Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Honolulu, HI 96822, USA

Giant leucaena can be grown over a range of pHs between 5.0 and 9.0, although it grows best at pHs between 6.0 and 8.0 (Blair et al. 1988; Hutton and Andrew 1978). Also, it was reported that under saline conditions leucaena can survive but the growth rate is reduced by 25–50% (Giles et al. 2014). The saline areas in the world are increasing at a rate of 10% annually for various reasons, including low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices (Jamil et al. 2011). Both pH and salinity may also affect the nutritional quality of giant leucaena foliage. Similarly, it is not known if the nutritional quality of the leucaena foliage changes with age of the leaves.

Giant leucaena can be grown as pasture legume where cattle are allowed to graze directly on the standing plants (Jones 1994). Alternatively, leucaena foliage is harvested and brought to the barn for feeding the cattle. In the later practice, there is always a time gap between harvesting and feeding, during which the nutritional quality may change. Therefore, it is important to study the effects of post-harvest storage time on the nutritional quality of giant leucaena foliage. Although giant leucaena is generally free from diseases and insect pests, some varieties are often infected by psyllids during early spring (Palmer et al. 1989). It is likely that psyllid infestation affects both yield and nutritional quality of young foliage that is the most palatable and nutritious part of the fodder. The goal of this investigation was to determine the effects of soil pH and salinity, age of the foliage, post-harvest storage duration, and psyllid infestation on the nutritional quality of giant leucaena foliage. The experiments were based on the hypothesis that soil pH and salinity would affect both fodder yield and quality, while age of leaves, psyllid infestation, and post-harvest duration would affect mostly the quality of the foliage (Fig. 1). The nutritional quality of the foliage was assessed by measuring the concentrations of crude protein, mimosine, total tannin, and structural fibers.

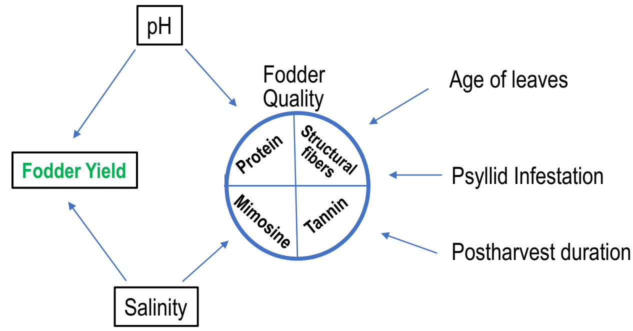

Fig. 1 The experiments in the present study were based on the hypothesis that soil pH and salinity would affect both fodder yield and quality, while age of leaves, psyllid infestation, and post-harvest duration would affect mostly the quality of the foliage. The fodder quality was assessed on the basis of concentrations of protein, mimosine, tannin and structural fibers in the foliage. High-quality leucaena fodder contains high concentration of protein (~18%), low concentrations of mimosine (~1%) and tannin (<5%), and medium amounts structural fibers (~40%)

Materials and methods

Giant leucaena grown in different pH conditions

Giant leucaena K636 seedlings were grown in the greenhouse in large pots containing potting mix planting soil (Sam's Club) for one year. Thirty of these 1-year-old plants, uniform in size, were selected for this experiment. The selected plants were then pruned so that they can develop fresh foliage uniformly. These plants were grouped into five pH groups (pHs 5, 6, 7, 8 and 9) and irrigated with diluted nutrient solutions adjusted to different pHs according to groups. The plants were irrigated on every other day for 13 weeks with water containing 2 g/L Miracle Gro™. After that, the green foliage of each plant was harvested, weighted, and used to quantify protein, mimosine, tannin and structural fibers.

Giant leucaena grown in different saline conditions

Thirty-two seedlings of giant leucaena K636, nine months in age, that were initially grown under the same condition as described above were used for this experiment. These plants were classified into four salinity groups. As in the previous experiment, these plants were also pruned to obtain uniform new shoots. The plants of different salinity groups were irrigated on alternate days with diluted nutrient solution (2 g/L Miracle Gro™) containing different salt concentrations (zero, 50, 100 and 150 mM NaCl). After 13 weeks, the green foliage of these plants was harvested, weighed, and used to quantify protein, mimosine, tannin and structural fibers.

Nutritional qualities of giant leucaena leaves of different maturity levels

To determine how the nutritional qualities of foliage may change with maturity, young, medium and old leaves were collected from four previously grown giant leucaena plants at the Waimanalo Research Center. Selection of young, medium and old leaves was based on color, rigidity and location of the leaves on the branch (Fig. 2). The young leaves are soft and tender, small in size, have a light green color, a green stem and are located at the branch tip. The medium aged leaves have a green color, rigid shape with a green stem and located in the middle of the branch. The old leaves have a dark green color, rigid shape with a brown stem and located towards the base of the branch

(Fig. 2). These three groups of leaves were harvested and used to quantify protein, mimosine, tannin and structural fibers.

Effects of post-harvest storage period on nutritional qualities of giant leucaena foliage

Four different giant leucaena plant samples collected from the Waimanalo Research Station were used in this experiment. Only medium aged leaves were harvested from these plants, and stored at room temperature (25 °C) in the laboratory. From these bulk samples, sub-samples were taken every 12 h for 3 days, to quantify protein, mimosine, tannin and structural fibers.

Psyllid infection

Psyllid-infected and uninfected giant leucaena leaf samples were collected from the Waimanalo Research Station (Fig. 3). The amounts of protein, mimosine, tannin, and structural fiber were quantified from these samples.

Protein quantification

Protein content of the leaf samples were determined using Bradford's method. Briefly, 300 mg fresh weight of leaf sample was grinded in liquid nitrogen. Then, 1 mL 0.1 N NaOH pH 12.8 was added to the samples, and vortexed harshly for 20 s before they were spun for 5 min at 7500 g. The supernatants were transferred to new 1.5 mL tubes and

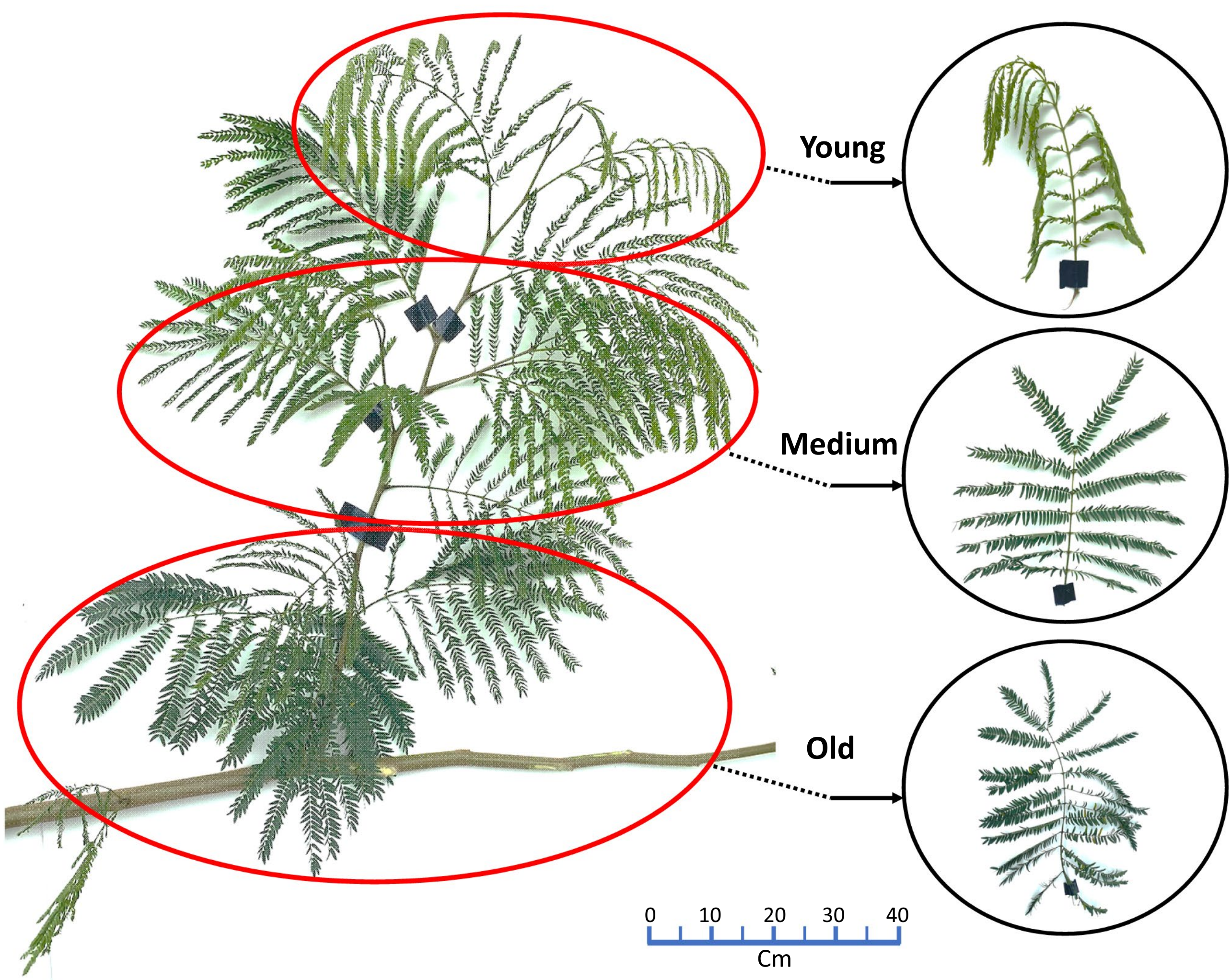

Fig. 2 Young, medium and old aged leaves are shown on a branch of giant leucaena. Young leaves have a light green color, small, soft, and loose leaflets, light green soft stems, and located at the branch tip. Medium-aged leaves have a darker green color, a more rigid leaflet's shape, and a green stem and located in the middle of the branch. Old leaves have a dark green color, rigid shape, a brown stem, and are located in the bottom of the branch. They also differ in chlorophyll **a**, chlorophyll **b** and carotenoids contents (see Fig. 6e)

centrifuged at 21,600*g* for 5 min. Then, the supernatants were transferred to new 1.5 mL tubes, to which an equal amount of 0.1 N NaOH pH12.8 was added, and mixed well. Thereafter, 100 µL of the mixture (supernatant + 0.1 N NaOH) was added to 4.9 mL of 1:4 diluted Bradford dye reagent, which contains 3 mg/mL polyvinylpyrrolidone (PVP), and the absorbance readings were recorded at 595 nm after 15 min incubation.

For dry sample protein quantification, fresh foliage samples, representing young, medium and old leaves, were collected from four giant leucaena trees. Twelve fresh leaf samples, 5 g each, were placed in the oven at 55 °C for 24 h to dry. The dry foliage (DW) was weighted, and the ratio of DW/FW was calculated. The protein contents of the samples were determined as described above.

Mimosine quantification

One g of freshly collected leaf sample was submerged in 20 mL 0.1 M HCl overnight with shaking. Thereafter, the plant debris was removed by centrifuging the plant extracts at 15,000*g* for 10 min. Mimosine concentration in a sample was determined by means of high-performance liquid chromatography (Waters 2650) with a C_{18} column and UV detection at 280 nm, using an isocratic carrier solvent of 0.02 M *O*-phosphoric acid and a linear flow rate of 1 mL per min for 6 min (Negi et al. 2014).

Tannin quantification

For total phenol extraction, 1 g of leaf sample was left in the oven overnight at 50–52 °C to dry. Then, 200 mg of the dried leaves were grinded well and placed in a 50 mL falcon tube. Ten mL of aqueous acetone (70%) was added to each sample and subjected to ultrasonic treatment at 100 Watt for 5 min. After that, the falcon tubes were centrifuged at 3000*g* for 10 min at 4 °C to remove plant debris. The supernatant was then collected and kept on ice. In 2 mL tubes, 10 µL of the supernatant was diluted in 490 µL dH_2O (50x) dilution. In addition, 250 µL of the Folin–Ciocalteu reagent (1 N) and 1.25 mL of sodium carbonate (20%) solution were added to the tube making a total of 2 mL in each sample tube. The tubes were incubated for 40 min under dark conditions, and finally absorbance readings were recorded at 725 nm.

For total tannins extraction, 1 mL of the supernatant was placed with 100 mg/mL of polyvinylpolypyrrolidone (PVPP) in a 2 mL tube, mixed well and placed at 4 °C for 15 min. After that, the tube was vortexed again, and spun at 3000*g* for 10 min. The supernatant represents the non-tannin phenolics, whereas tannin was bound to PVPP. After that, 50 µL of the supernatant was diluted in 450 µL dH_2O (10x) dilution. The rest of the steps were the same as described for total phenolics. Finally, the concentration of total tannin was calculated by subtracting total phenolics from non-tannin phenolics (Makkar 2003).

Structural fibers percentage

Total structural fibers was quantified by measuring the neutral detergent fibers (NDF). Dried leaf samples (0.5 g) were placed in the filter bags. Two liters (100 mL/bag) of Neutral Detergent solution was added into the fiber analyzer vessel ANKOM 200/220 module with 20 g (0.5 g/50 mL of ND solution) of sodium and 4.0 mL of heat stable alpha-amylase. The

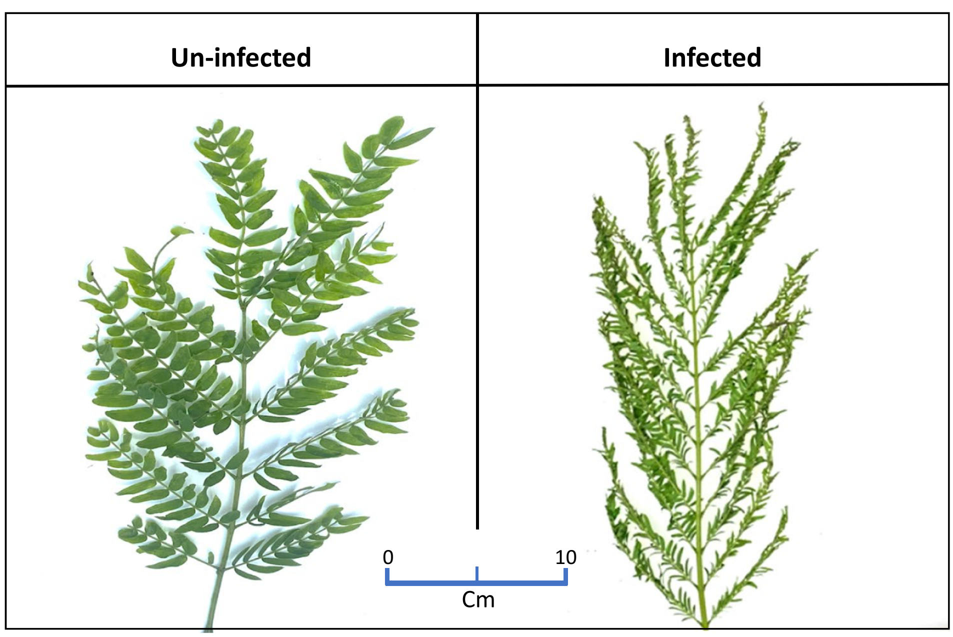

Fig. 3 Visible signs of psyllid infection on medium aged leaves of giant leucaena foliage. The differences between uninfected and psyllid-infected leucaena leaves are shown. Uninfected leaves have un-crinkly and un-sticky leaves, whereas infected leaves have crinkly and sticky leaves

structural fibers were quantified using the protocol for neutral detergent fibers quantification as described in the manual for ANKOM 200/220 Fiber Analyzer (ANKOM Technology, Macedon NY).

Chlorophyll quantification

For pigments quantification, 1.5 mL of *N*-dimethylformamide was added to 15 mg of fresh leaves, and left in the dark room for 3 days. After that, samples were vortexed at slow speed for 1 min, and centrifuged for 5 min at 13,500*g*. Finally, the extract was placed in the spectrophotometer, and read at three different wavelengths (A664, A647 and A480). The calculations for chlorophyll a, chlorophyll b, and carotenoid were done according to (Minocha et al. 2009) using the equations below:

$$\text{Chlorophyll a} = (12 * \text{A664}) - (3.11 * \text{A647}),$$

$$\text{Chlorophyll b} = (20.78 * \text{A647}) - (4.88 * \text{A664}),$$

$$\text{Carotenoid} = (1000 * \text{A480} - 1.12\text{Ch a} - 34.07\text{Ch b})/245.$$

Results

Effects pH on biomass production and nutritional qualities of giant leucaena

To determine the effects of soil pH on leucaena fodder productivity, giant leucaena K636 was grown in the greenhouse under five different pHs ranging 5.0–9.0. After growing these plants for 13 weeks, the aerial parts were harvested and data were recorded for biomass and concentrations of protein, mimosine, tannin, and total structural fibers (NDF). Biomass production did not fluctuate much from pH 6.0–8.0 and was much lower at both pHs 5.0 and 9.0 (Fig. 4a). Protein concentration of the foliage also followed a similar trend, with the highest protein concentrations at pH 6.0 and lower concentrations at both pH 5.0 and at pH 9.0 (Fig. 4b). The mimosine concentrations were highest at pHs 6.0 and 7.0 but were lowest at pHs 5.0 and 9.0 (Fig. 4c). Tannin concentrations of the foliage were not affected by pH (Fig. 4d). Although total structural fiber production was highest at pH 6.0, it did not fluctuate much at lower or higher pHs (Fig. 4e).

Effects salinity on biomass production and nutritional qualities of giant leucaena

To determine the effects of soil salinity on leucaena fodder productivity, giant leucaena K636 was grown in the greenhouse under four different salt concentrations zero, 50, 100 and 150 mM NaCl. After growing the plants for 10 weeks, the aerial parts of the plants were harvested and data were recorded for total biomass and concentrations of crude protein, mimosine, tannin, and total structural fibers (NDF). Plants that were planted in 150 mM NaCl did not survive at all. Biomass production is reduced with increases in salt concentrations (Fig. 5a). Protein concentrations of the foliage reduced with increased concentrations of salt in the soil (Fig. 5b). In addition, both mimosine and tannin concentrations decreased in plants with rising soil salinity (Fig. 5c, d). Total structural fiber production did not fluctuate much in the three different treatments (Fig. 5e).

Effects of age of leaves on nutritional properties of giant leucaena foliage

To evaluate if the nutritional properties of giant leucaena leaves change with maturity, leaves representing three maturity groups were collected and analyzed. Young leaves contain the highest amount of moisture (76.8%) and least amount of dry matter (23.2%) (Table 1). The concentrations of protein, mimosine, tannin, total structural fibers (NDF), and photosynthetic pigments (chlorophylls and carotenoids) were recorded in these leaves at different maturity stages. Protein contents were at their highest when leaves were young, and got reduced with maturity (Fig. 6a, Table 1). Young leaf samples were found to contain the highest concentrations of mimosine, whereas the oldest leaf samples contained the lowest (Fig. 6b). On the other hand, tannin concentrations of young and medium aged leaves were similar, and reduced with maturity (Fig. 6c). In contrast, total structural fiber production increased as leaves were getting older (Fig. 6d). For pigment quantities, as the leaves were getting older, they accumulated more pigments (chlorophyll a and b, and carotenoids) (Fig. 6e).

Effects of storage of harvested giant leucaena leaves for an extended period

Medium-aged leaf samples were collected from giant leucaena K636 grown at the Waimanalo Research Station. The concentrations of protein, mimosine, tannin, and total structural fibers (NDF) were recorded at different times following harvest (Fig. 7). The only parameter that has changed over time was mimosine, which started to reduce

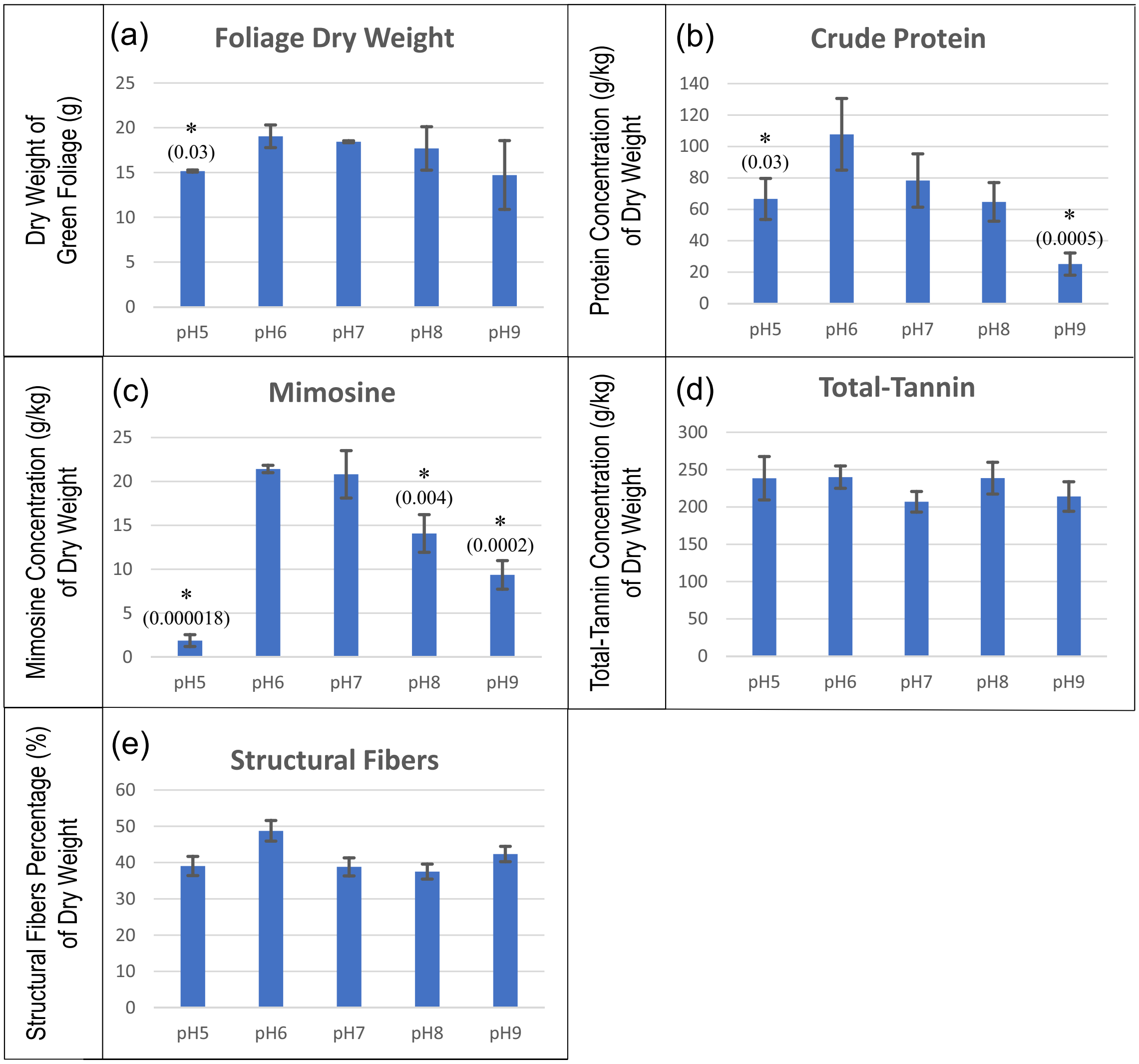

Fig. 4 Comparison among medium aged leaves of giant leucaena plants grown for 13 weeks at different pH for (**a**) green foliage dry weight, (**b**) crude protein, (**c**) mimosine, (**d**) total tannin and (**e**) structural fiber contents. The error bars in (**a**) indicate ± SE ($n = 3$ biological replicates). The error bars in (**b**), (**c**), (**d**) and (**e**) indicate ± SE ($n = 9$, 3 biological replicates, each having 3 technical replicates). Significant differences from the mean of the highest reading are shown by single asterisks and corresponding p values above the error bars

after 48 h (Fig. 7b). On the other hand, all other parameters (crude protein, tannin and structural fibers) did not change within 72 h of post-harvest storage (Fig. 7a, c, d).

Effects of psyllid infestation on nutritional properties of giant leucaena

To determine if psyllid infection affects nutritional quality of giant leucaena, infected and uninfected leaf samples were compared for protein, mimosine, tannin, and structural fiber contents. Protein and structural fiber contents were not much affected, whereas mimosine and tannin contents were greatly increased (77% and 68%, respectively) as a result of psyllid infection (Fig. 8).

Discussion

Giant leucaena is an ideal fodder for several reasons: (i) it gives high green fodder yield, (ii) it has high protein content, (iii) it is highly palatable to farm animals, (iv) it can be grown under a wide range of tropical and subtropical environmental conditions, (v) it has high capacity to regenerate following pruning, and (vi) it is highly responsive to favorable growth conditions, such as irrigation and fertilizer. Although the presence of mimosine in the leucaena foliage is undesirable, it is not considered to be a serious problem. The problem of mimosine toxicity of leucaena has been addressed in various ways, including use of ruminal bacterium *Synergistes jonesii* as an oral inoculum (Jones and

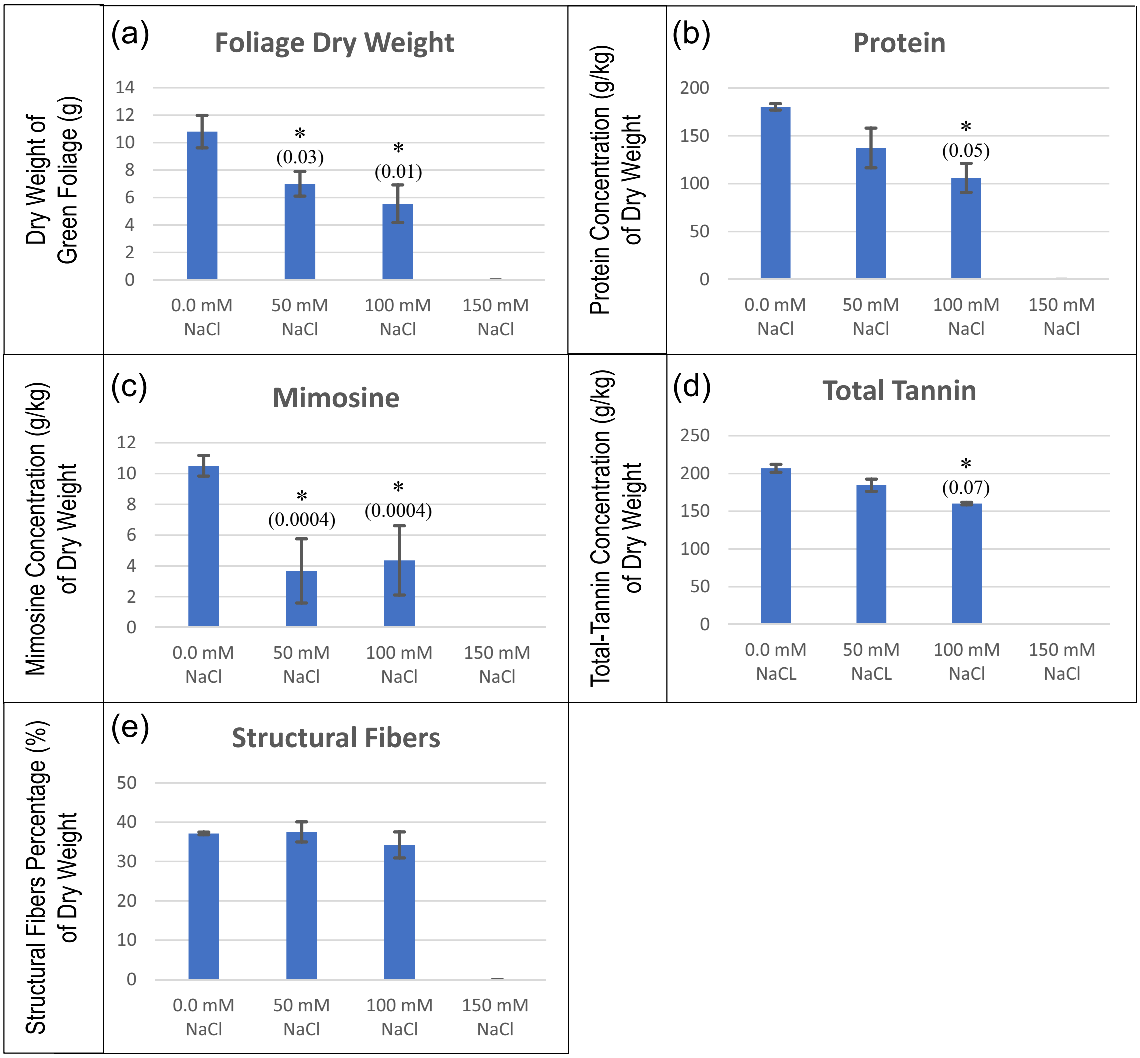

Fig. 5 Comparison among medium aged leaves of giant leucaena plants grown for 13 weeks treated with different levels of salinity for (**a**) green foliage dry weight, (**b**) crude protein, (**c**) mimosine, (**d**) total tannin and (**e**) structural fiber contents. The error bars in (**a**) indicate ± SE (n = 4 biological replicates). The error bars in (**b**), (**c**), (**d**) and (**e**) indicate ± SE (n = 12, 4 biological replicates, each having 3 technical replicates). Significant differences from the mean of the highest reading are shown by single asterisks and corresponding p values above the error bars

Table 1 Dry matter and protein contents of young, medium, and old leaves of giant leucaena

Age of leaves	Fresh weight (FW) (g)	Dry weight (DW) (g)	Dry matter (%)	Moisture (%)	Protein in FW (%)	protein in DW (%)	FW/DW Protein ratio (%)
Young	5.05 ± 0.006	1.16 ± 0.019	23.2 ± 0.39	76.8 ± 0.39	4.2 ± 0.27	18.2 ± 1.5	23.1 ± 1.13
Medium	5.08 ± 0.028	1.17 ± 0.029	28.6 ± 0.61	71.4 ± 1.08	4.2 ± 0.19	14.6 ± 0.65	28.8 ± 0.1
Old	5.05 ± 0.019	1.13 ± 0.045	28.05 ± 0.86	71.95 ± 1.54	3.6 ± 0.19	12.4 ± 0.67	28.85 ± 0.15

± Denotes standard error from four samples

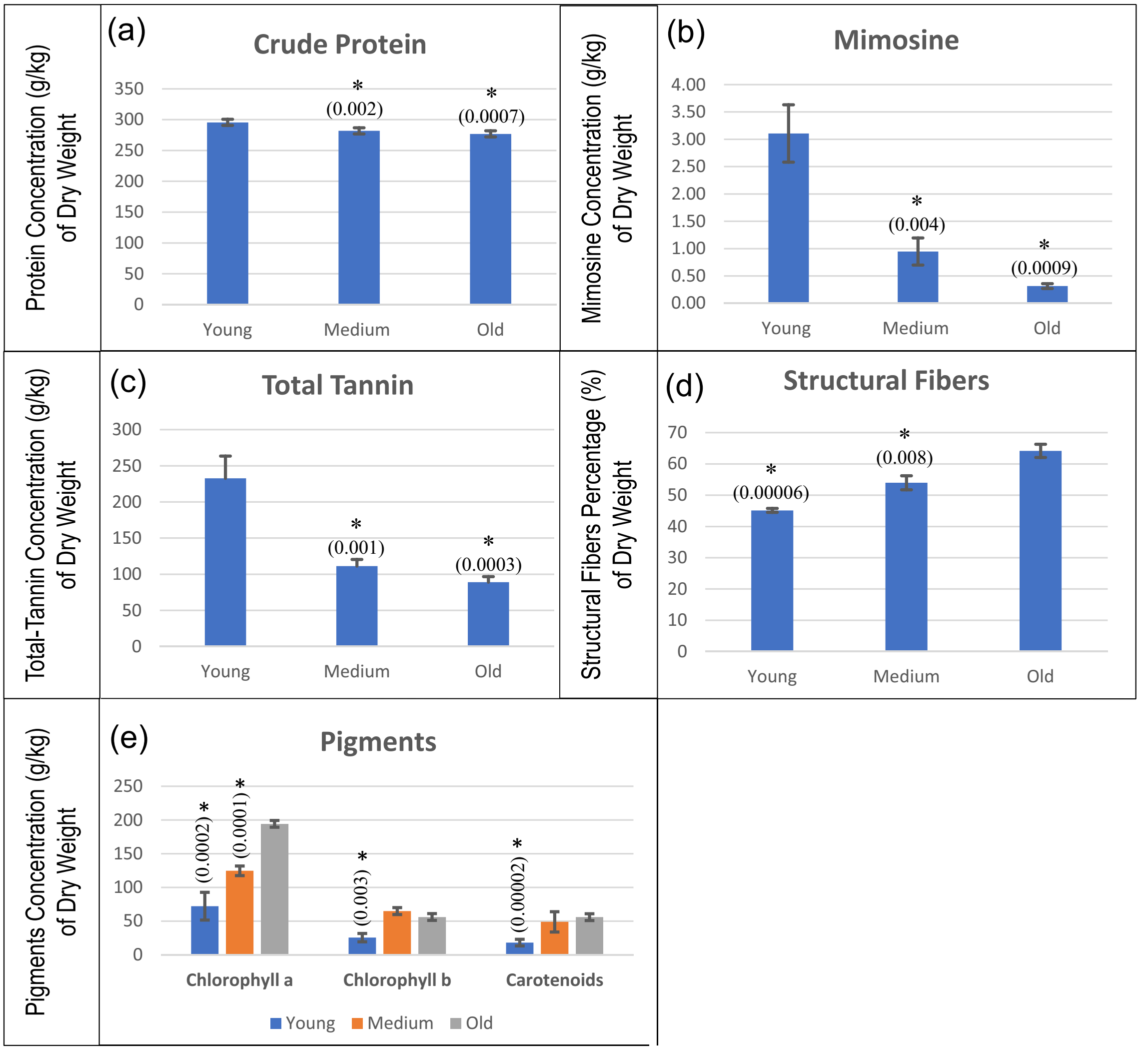

Fig. 6 Comparison among young, medium and old leaves of giant leucaena for (**a**) crude protein, (**b**) mimosine, (**c**) total tannin, (**d**) structural fiber, and (**e**) pigments (chlorophyll a, chlorophyll b and carotenoids) contents. The error bars indicate ± SE ($n = 12$, 4 biological replicates, each having 3 technical replicates). Significant differences from the mean of the highest reading are shown by single asterisks and corresponding p values above the error bars

Megarrity 1986), through selection of leucaena accessions for lower mimosine content (Brewbaker 2016), through genetic engineering of leucaena (Jube and Borthakur 2010), and feeding leucaena in combination with grasses (Tanner et aI. 1995). There is also a lot of variability among ruminants for their ability to detoxify mimosine (Halliday et al. 2013). Some ruminants in Indonesia have another pathway for mimosine detoxification, in which 2,3-DHP, a degradation product of mimosine is released with urine in a conjugated form with glucuronic acid (Shelton et al. 2019). The emphasis of the present investigation is determining the effects of certain environmental factors and age of leaves on nutritional quality of leucaena foliage, including its mimosine content. Although leucaena foliage has high nutritional values, they can be influenced by environmental factors like pH, salinity, and psyllid infestation. Similarly, the age of the foliage and the post-harvest storage duration may have some effects on the nutritional qualities of giant leucaena foliage. This investigation was aimed in elucidating some of these factors affecting the nutritional qualities of giant leucaena

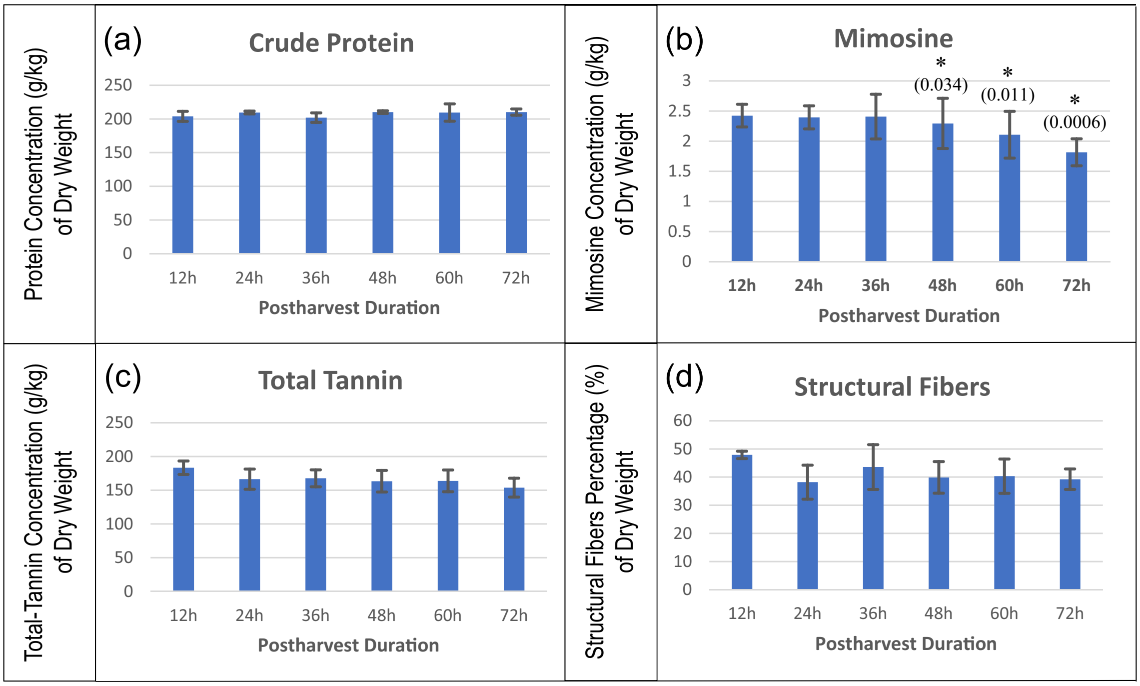

Fig. 7 Medium-aged leaves of post-harvest (**a**) crude protein, (**b**) mimosine, (**c**) total tannin and (**d**) structural fiber contents of giant leucaena foliage, stored at room temperature for different lengths of time after harvest. The error bars indicate ± SE ($n = 12$, 4 biological replicates, each having 3 technical replicates). Significant differences from the mean of the highest reading are shown by single asterisks and corresponding p values above the error bars

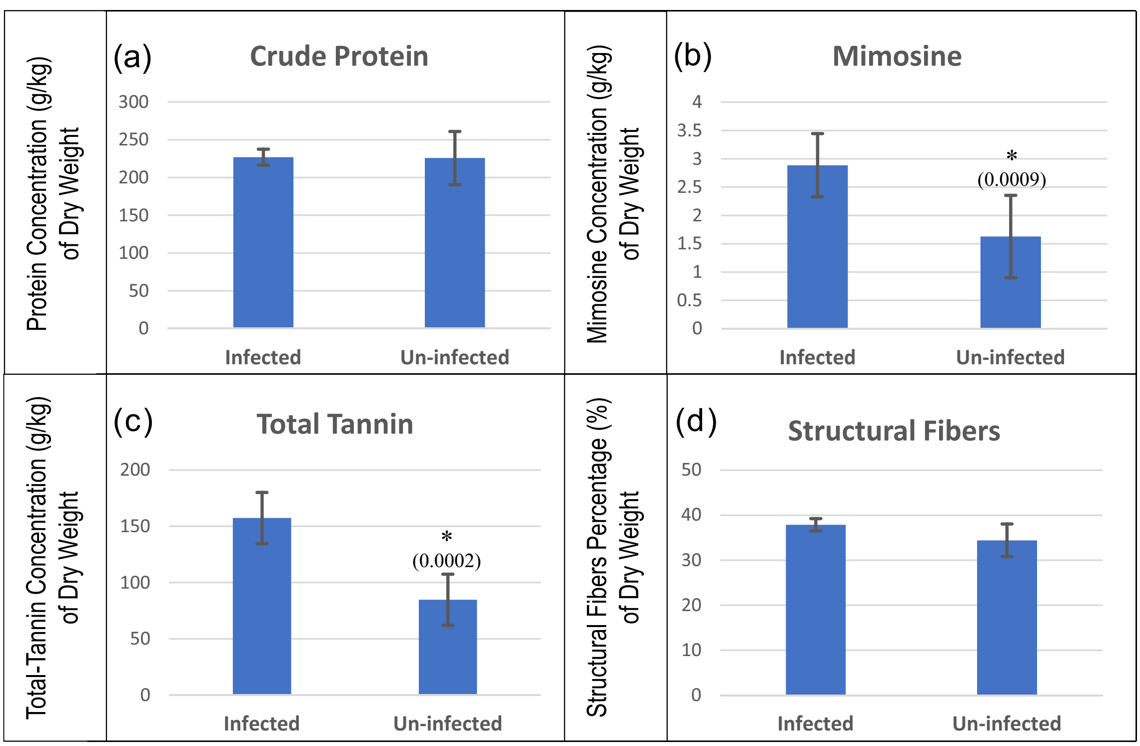

Fig. 8 **a** Crude protein, **b** mimosine, **c** total tannin and **d** structural fiber contents of healthy (uninfected) and psyllid-infected giant leucaena foliage young leaves. The error bars indicate ± SE ($n = 12$, 4 biological replicates, each having 3 technical replicates). Significant differences from the mean of the highest reading are shown by single asterisks and corresponding p values above the error bars

foliage. In this study, besides protein content, a few other parameters, including mimosine, tannin, and total structural fiber, were measured as indicators for nutritional quality.

Most of the reports of the protein contents in the literature were based on dry weights, and the general range of the protein content was found to be 20–30% (Ekpenyong 1986; Garcia et al. 1996; Jones 1979; Norton 1994; Wheeler et al. 1994). Moreover, most reports estimated protein content on the basis of total nitrogen content that includes the nitrogen present in mimosine. Soedarjo and Borthakur (1998) observed that the amount of protein in the leucaena foliage might be overestimated due to the presence of mimosine in the samples and suggested an alternative method for accurate estimation of protein amount. The reagents used in Lowry's method cross-reacted with mimosine resulting in an overestimation of the protein amount. In the present study, Bradford's method was used for estimation of protein amount and it was verified that the presence of mimosine in the samples does not interfere with the protein estimation in this method (Fig. S-1). Although the protein concentrations were shown mostly on a dry weight basis, in one example, both fresh and dry weights were shown for a comparison (Table 1).

In spite of having high protein contents, leucaena foliage has some undesirable secondary metabolites such as mimosine and tannin. Generally, giant leucaena fodder is fed to animals as a protein supplement along with grass or hays such that mimosine consumption stays below 0.18 g/kg of body weight, above which it can be harmful to animals. Similarly, high concentration of tannin in fodder interferes with protein uptake (Shahkhlili et al. 1990). Usually, less than 50 g/kg of dry matter is considered safe and not harmful to animals (Tanner et aI. 1995). Another major consideration in using legume fodder is its total structural fiber content; generally, high fiber content is associated with low nutrient uptake. The desirable range of total structural fiber content that does not significantly inhibit nutrient uptake is 35–55% of dry weight (Prajapati et al. 2018).

To identify the most ideal pH condition suitable for obtaining the highest nutritional value of leucaena foliage, it is necessary to consider all quality parameters together. Among various parameters, protein content is considered the most important for determining the nutritional value of the fodder. In the present study, both fodder and protein yields were highest at pH 6.0. In addition, mimosine and total structural fibers contents were highest at pH 6, and greatly reduced at pH 5. On the other hand, tannin was not affected by soil pH. Generally, the ideal soil pH for growing leucaena is known to be close to neutral, which can range from a slightly acidic pH of 6.5 to a slightly alkaline pH of 7.5 (Blarney and Hutton 1995; Oakes and Foy 1984). At lower pHs (≤ pH5), nutrients such as molybdenum and calcium are not easily available, resulting in lower growth of most plant species, including giant leucaena. On the other hand, at higher pHs (≥ 8.0), other nutrients such as iron and zinc are only sparsely available to plants. In the present study, although a soil pH of 6.0 was found to be the best among the pHs tested for both growth and protein yield, it was not ideal for reducing the amounts of mimosine, tannin and total structural fiber of giant leucaena.

Based on the data, although giant leucaena can be grown at salt concentration of up to 100 mM, the growth was retarded in the presence of any amount of salt. Total green fodder yield, and protein and mimosine contents were drastically reduced with increasing salt content. These results indicate that giant leucaena is not suitable for growing in saline soils. Other researchers also observed the inhibitory effects of salt on growth and fodder yield of giant leucaena (Brewbaker 1987).

The leaves of leucaena plants can be divided into three age groups; young, medium and old. When giant leucaena is grown for fodder, the plants are maintained as shrubs by repeated harvest of its foliage every 2–4 months. If it is harvested frequently, e.g., at intervals of two months, the foliage will contain mostly young and moderately old leaves, but if the harvesting intervals are prolonged, e.g., four months, the foliage will contain some old leaves also. It is likely that the nutritional quality of the foliage will depend on the age of the foliage. To determine if the age of the leaves affects nutritional qualities, young, moderately old and old leaves were harvested and their protein, mimosine, tannin and total structural fibers were quantified. With age, protein, mimosine and tannin contents of the leaves decreased while structural fiber contents increased. The decrease in the protein content in the old leaves compared to the young leaves was only 18.5%. Similarly, the decreases in mimosine and tannin contents in the old leaves compared to the young leaves were 95% and 30%, respectively. On the other hand, the old leaves had 40% more structural fibers than the young leaves. Decreases in mimosine and tannin are desirable while decrease in protein content and increase in structural fibers are not desirable for fodder quality. Therefore, although prolonged harvesting intervals of giant leucaena foliage may improve fodder quality by reducing mimosine and tannin contents, it will have some negative impacts due to slight reduction in protein content and increase in structural fibers.

Following harvest, the foliage of giant leucaena may be fed to animals immediately or stored for a few days for feeding the animals. To determine if there are any effects of storage on nutritional properties of the giant leucaena fodder, the foliage was analyzed after harvesting for protein, mimosine, tannin and total structural fiber every 12 h for three days. The amounts of protein, tannin and structural fibers did not change much due to storage up to 72 h. Mimosine concentration started to decrease from the second day onwards, and on the third day, the foliage lost 25% of mimosine. It is known that leucaena contains a mimosine-degrading enzyme, mimosinase, in the chloroplast (Negi et al. 2014). It is possible that during storage, some chloroplasts break down releasing mimosinase into the cytoplasm, where it degrades mimosine. Thus, post-harvest storage of giant leucaena foliage over 48 h, at room temperature may be used as a method to reduce mimosine content of leucaena fodder. In this study, the concentrations of structural fibers in young and the medium old leaves were found to be less than 55% of the dry weight, which is not considered inhibitory for nutrient uptake. However, the old leaves contained over 60% structural fiber, which reduces palatability and digestibility of the foliage.

In spite of the presence of both mimosine and tannin in leucaena, the plants were infected by psyllids. Interestingly, psyllid infection increased both mimosine and tannin contents of leucaena foliage. Therefore, it is likely that in the distant past, mimosine and tannin might have served as a defense mechanism against psyllid infestation, however, in the due course of time, the psyllids have developed mechanisms to overcome this defense. Kamada et al. (1995) Found that psyllid infesting on leucaena contained an enzyme to hydrolyze mimosine. It is possible that psyllids became tolerant to mimosine by acquiring a new microbial symbiont that can degrade mimosine.

Conclusions

Among various nutritional attributes of giant leucaena fodder, protein content is considered the most important. The protein content and biomass yield were found to be highest at pH 6.0, and at neutral pH these two parameters were slightly reduced. However, when leucaena was grown at pH 6.0, the structural fibers were also high. At this pH, giant leucaena green foliage contained about 3.8% mimosine, which could be further reduced by post-harvest storage of the foliage over 48 h. Protein content did not change much with the age of the foliage whereas both mimosine and tannin contents were much reduced when the leaves became older. The older leaves contain higher amounts of structural fibers. Therefore, for obtaining optimum benefits, the harvesting intervals should be such that the foliage is moderately mature when most of the leaves are neither too old nor too young. Such foliage of intermediate maturity is expected to contain relatively high protein content, low mimosine and tannin contents, and moderate structural fibers content. In addition, the storage of the foliage at room temperature for a few days may affect the nutritional quality of the foliage by reducing the quantity of mimosine.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12892-021-00139-9.

Acknowledgements AB was supported by a scholarship from the Saudi Cultural Mission, Virginia. This research was supported by a USDA McIntire grant.

Declarations

Conflict of interest The authors declare that they have no conflict of interest. They have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Bageel A, Honda M, Carrilo J, Borthakur D (2020) Giant Leucaena (*Leucaena leucocephala* subsp. *glabrata*): a versatile tree-legume for sustainable agroforesty. Agrofor Syst 94:251–268. https://doi.org/10.1007/s10457-019-00392-6

Blair GJ, Lithgow KB, Orchard PW (1988) The effects of pH and calcium on the growth of *Leucaena leucocephala* in an oxisol and ultisol soil. Plant Soil 106:209–214

Blarney FPC, Hutton EM. 1995. Tolerance of Leucaena to acid soil conditions. In: Shelton, H.M.; Piggin, C.M. and Brewbaker, J.L. (ed) Leucaena: Opportunities and limitations. Proceedings of a Workshop held in Bogor, Indonesia, January 1994. ACIAR Proceedings 57, Canberra, pp 83–86

Brewbaker JL (2016) Breeding Leucaena: tropical multipurpose leguminous tree. Plant Breed Rev 40:43–121

Brewbaker JL, Plucknett DL, Gonzalez V. 1972. Varietal variation and yield trials of *Leucaena leucocephala* (Koa Haole) in Hawaii. Research Bulletin, Hawaii Agricultural Experiment Station, No. 166, 29pp. https://scholarspace.manoa.hawaii.edu/bitstream/10125/40996/1/varietalvariation.pdf

Brewbaker JL (1987) Leucaena: a multipurpose tree genus for tropical agroforestry. In: Steppler, HA, Nair, PK (ed) Agroforestry: a decade of development. international council for research in agroforestry. Nairobi, Kenya, pp 289–323

Ekpenyong TE (1986) Nutrient and amino acid composition of *Leucaena leucocephala* (lam.) de wit. Anim Feed Sci Technol 15:183–187

Elfeel AA, Elmagboul AH (2016) Effect of planting density on *Leucaena leucocephala* forage and woody stems production under arid dry climate. Int J Env Agri Res 3:7–11

Garcia GH, Ferguson TU, Neckles FA, Archibald KAE (1996) The nutritive value and forage productivity of *Leucaena leucocephala*. Anim Feed Sci Tech 60:29–41

Hutton EM, Andrew CS (1978) Comparative effects of calcium carbonate on growth, nodulation and chemical composition of four legumes. Aust J Exp Agric 18:81–88

Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. Crit Rev Plant Sci 30:435–458

Jones RJ (1979) The value of *Leucaena leucocephala* as a feed for ruminants in the tropics. Worl Anim Rev 31:13–23

Jones RJ (1994) Management of anti-nutritive factors - with special reference to leucaena. In: Gutteridge RC, Shelton HM (eds) Forage tree legumes in tropical agriculture. CAB International, Wallingford, Oxford, UK, pp 216–231

Jones RJ, Megarrity RG (1986) Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. Aust Vet J 63:259–262

Jube SLR, Borthakur D (2010) Transgenic *Leucaena leucocephala* expressing the *Rhizobium* gene *pydA* encoding a meta-cleavage dioxygenase shows reduced mimosine content. Plant Physiol Biochem 48:273–327

Kamada Y, Oshiro N, Oku H, Hongo F, Chinen I (1995) Degradation of toxic mimosine by a hydrolytic enzyme of leucaena psyllids (jumping plant lice). Biosci Biotechnol Biochem 60:351–352

Makkar HPS. 2003. Measurement of total phenolics and tannins using folin-ciocalteu method. In: Quantification of tannins in tree and shrub foliage. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0273-7_3

Minocha R, Martinez G, Lyons B, Long S (2009) Development of a standardized methodology for quantifying total chlorophyll and carotenoids from foliage of hardwood and conifer tree species. Can J for Res 39:849–861

Negi VS, Bingham JP, Li QX, Borthakur D (2014) A carbon-nitrogen lyase from *Leucaena leucocephala* catalyzes the first step of mimosine degradation. Plant Physiol 164:922–934

Norton BW (1994) The nutritive value of tree legumes. In: Gutteridge RC, Shelton HM (eds) Forage tree legumes in tropical agriculture. CAB International, Wallingford, Oxford, UK, pp 177–191

Oakes AJ, Foy CD (1984) Acid soil tolerance of Leucaena species in greenhouse trials. J Plant Nutr 7:1759–1774

Palmer B, Fulloon MG, Bray RA, Ibrahim TM (1989) The effect of the Leucaena psyllid on the yield of *Leucaena leucocephala* cv. *Cunningham* at four sites in the tropics. Trop Grassl 23:105–107

Prajapati B, Tiwari S, Kewalanand. 2018. Effect of fodder based intercropping systems on quality of fodder. Forage Res. 43: 308-313

Soedarjo M, Borthakur D (1998) Mimosine, a toxin produced by the tree-legume Leucaena provides a nodulation competition advantage to mimosine-degrading Rhizobium strains. Soil Biol Biochem 30:1605–1613

Szyszka M, Ter Meulen U (1984) The acceptable daily intake for mimosine in productive livestock and its importance for the use of *Leucaena leucocephala* in animal nutrition. Dtsch Tieraerztl Wochenschr 91:260–262

Tanner GJ, Moate P, Dailey L, Laby R, Larkin PJ (1995) Proanthocyanidins (condensed tannins) destabilise plant protein foams in a dose dependent manner. Aust J Agric Res 46:1101–1109

Wheeler RA, Chaney WR, Cecava MJ, Brewbaker JL (1994) Forage yield and compositional analysis of Leucaena species and hybrids adapted to cool sites. Agrofor Syst 25:263–274

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.