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Allelopathic Effect of *Melia azedarach* L. and *Populus nigra* L. on Germination and Growth *Brassica campestris* L.

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ABSTRACT

Brassica campestris is a typical daily crop grown all over the world as a source of fodder, vegetables, and oil. The allelopathic effects of *Populus nigra* L. and *Melia azedarach* L. extracted leaves on seed germination and growth performance of *Brassica campestris* L. were studied in the field and in the laboratory. Seeds of *Brassica campestris* were seeded in pots in the field, whereas seeds sown in tap water were considered as a control. Different leave weight, of both *P. nigra* L. and *M. azedarach* was used and mixed with soil separately, to test their allopathic effects. Growth parameters including stem height, stem diameter, leaf number, number of internodes, internodal length, fresh and dry weight of the stem, and 1000 seed-weight were investigated. Number of flowers per plant; and inflorescence size were also considered. The results revealed that all measured parameters of *B. campestris* were negatively influenced, with the exception of internodal length, which was found to be positively affected. Seeds treated with plant extracts showed an inhibitory effect on seed germination which was directly proportional with doses of leaves weight used. In laboratory experiments, the allelopathic effects of *P. nigra* aqueous extract on seed germination (%) as well as growth performance of germinated seed, expressed as plumule length and radicle length, of *B. campestris* were reported. Parallel to the field experiment, a high dose of leaf weight extracted from dried leaves greatly decreased seed germination (%) and growth measured parameters, which are proportional to extracted leaf weight.

Keywords: Allopathy treatment; *Brassica campestris; Populus nigra* L.; *Melia azedarach* L.; Phenolic compounds.

INTRODUCTION

Allelopathy is a term used to describe the study of plant chemical interactions. The term is derived from two Greek words: "allelon," which means "opposite," and "pathos," which means "to suffer. This word was coined by German physician Samuel Hahnemann in the 1800s. Indeed, as time passed, this phrase came to symbolize a phase of symptom reduction. These allelopathic chemical compounds are released by living organisms and have an influence on the health and survival of other living things (Iqbal *et al*., 2020). In higher plants, these substances are located in the root, leaf, stem, flower, and fruit, and they are released under certain conditions. They have an impact on both soil bacteria and other plants in the vicinity, influencing seed germination, root and stem growth, and other plant functions (Yu *et al*., 2003; Iqbal *et al*., 2020). Generally, allelopathy is an ecological process that involves the release of secondary metabolites into the environment, which has both positive and negative consequences between organisms. The production of these compounds is highly influenced by different phases of plant growth, genetics and environmental variables (Yu *et al*., 2003). Furthermore, the concentrations of these allelochemicals in the generating plant and the plant tissue produced may change over time. For example, foliar and leaf litter leachates of *Eucalyptus* species, are more toxic than bark leachates to some food crops (Singh and Kumar, 2009). A large number of plants impose inhibitory effects on the germination and growth of neighboring or successional of plants by releasing allelopathic chemicals into the soil, either as exudates from living tissues or by decomposition of plant residues (Khan *et al*., 2009).

The phenomenon of allelopathy has received increasing attention as a means of explaining veget-ation patterns in plant communities (Amir *et al*., 2018). Allelopathy may occur in all environments and should be considered as a part of community interaction (Inderjit *et al*., 1999). Allelopathy plays an important role in many agroecosystems (Singh *et al*., 2008). Generally, the degree of allelopathic inhibition

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increases with increasing extract concentrations (Laosinwattana *et al*., 2010). Most of the initial work performed with laboratory bioassays of allelochemicals had generally focused on seed germination and seedling growth (Oraon and Mondal, 2020). The interactions between trees and crops in agroforestry, the trees may have a significant bearing on crop production under integrated land use systems rather than mono agriculture. There may also be competition for light, soil moisture and nutrients between trees and crops. However, utilization methods of growth response and biochemical effects (allelopathy) are equally interested in poor germination and growth of vicinity vegetation (Singh *et al*., 2008; Pezzopane *et al*., 2021).

Since *P.nigra* and *M. azedarch* are significant trees for soil rehabilitation, timber production, and field protection as shelter belts, it's important to consider the effect of their fallen leaves on the growth characteristics of the crop in the vicinity. Some studies have been done and showed an inhibitory effect of the aqueous extracts of *M. azedarach* at different concentations on seed germination and seedling growth of sesame, *Vigna radiata* L. and *Cicer arietinum* L. compared to control (Soleymani and Shahrajabian, 2012; Shahid *et al*., 2017). Therefore, the current research aims to examine the allelopathic effects of *P. nigra* and *M. azedarach* leaves extract on the seed germination, seedling growth, and other growth parameters of *B. campestris* at both fields and trials.

MATERIALS AND METHODS

Plant materials

The *B. campestris* seeds variety Abasyn was obtained from IBGE (Institute of Biotechnology and Genetic Engineering), The University of Agricultural, Peshawar Pakistan. The fresh leaves of *Melia azedarach* and *Populus nigra* were collected from the campus Islamia college Peshawar (2018-19). The leaves were washed with tap water and were dried in shade. These leaves were crushed and then utilized for further field and laboratory experiments.

Experimental Design

The study included two sets of experiments, field and laboratory experiments. The field experiment was conducted in an environmentally controlled experimental house at Islamia College in Peshawar, Pakistan. The Allelopathic effects of *M. azedarach* and *P. nigra* were tested against *B. campestris* in the field, using plastic pots (3 replicates for each treatments), and in the laboratory, using Petri dishes under control conditions (Temperature ranged from 20 °C to 25 °C with humidity levels between 30% and 50%).

Field experiments

Plastic pots were filled with soil after being mixed with 100, 200 and 300 g of *M. azedarach* and *P. nigra* L. crushed leaves, separately. For control, only soil was used. After then, *B. campestris* seeds were sown on early December 2018-19. Regular water irrigation was done after 15 days of sowing when the seeds have grown out. The Allelopathic of *M. azedarach* and *P. nigra* were evaluated per pot based on number of germinated seeds (%), stem height, stem diameter, number of leaves, leaf length, numbers of internodes, internode length, number of flowers and size of inflorescence as well as fresh and dry weight. Moreover, weight of 1000 of grains was also considered.

Laboratory Experiments

Effect *P. nigra* leaves on germination percentage, radical and plumule length of *B. compestris* seeds were investigated. In this experiment, fresh leaves of *P. nigra* were collected, and divided into two groups in which fresh leaves were weight and soaked separately in water. Second group was ground after being dried. For fresh and dry leaves used, 10, 15 and 20g were soaked separately in 100ml of distilled water and kept for 72hours and then filtered through Whatman filter paper. The filtrate was used to irrigate *B. campestris* seeds placed on a filter paper in glass Petri-dishes. Three replica for each treatment were carried out in which fives seeds of *B. campestris* were used. For control group, distilled water was used instead of filtrate. The Petri-dishes were incubated at 25 °C for 72 hours. Data were recorded after 72 hr.

Statistical analysis

The studies were set up with at least three replicates per treatment in a properly randomized manner. The results were subjected to ANOVA test using statistic 8.1 (2019). Data are represented in mean ±SE. Duncan’s Multiple Range Test was also performed to compare the significance of among means.

RESULTS

Allelopathic effect of *M. azedarach* and *P. nigra* leaves

Field experiment

Seed germination percentage

The data obtained showed an inhibitory effect of either leaves of *M. azedarach* or *P. nigra*, on percentage of seed germination of *B. campestris* L, when mixed with soil in pots for the field experiment. Germination of the seeds showed significant inhibition percentage compared to control for both tested plants. Meanwhile, this value increased with increment the amount of crushed leaves (Fig. 1A).

Stem height

M. azedarach or *P. nigra* dry crushed leaves inhibited *B. campestris* stem height compared to control pots (Fig. 1B). The data revealed that the inhibitory effect on stem height was significantly ($p \leq 0.05$) the highest at 300g dry leaves, with a reduction of 41.55 % compared to the control. Similarly, applying 300g dry crushed *P. nigra* leaves resulted in a 48.27 % decrease in stem height compared to the control, at $p \leq 0.05$ level (Fig. 1B). Similar results were obtained with plumule length by Khattak *et al.* (2016) who used the plant bark of *P. nigra* to explore its allelopathic effect on *Zea mays* under laboratory condition.

Stem diameter

Results obtained (Fig. 1C) also recorded an inhibitory effect on stem diameter of *B. campestris* when treated with dry leaves of the studied plants. *M. azedarch* had the highest reduction effect with highest weight of crushed dry leaves (pots containing 300g) compared to control (1.93, 3.33cm, respectively). In parallel, *P. nigra* had inhibitory effect on stem diameter but with less reduction and recorded 1.74 cm compared to control (3.14 cm). These results are in consistence with data obtained by Sun *et al.* (2006) who investigated the effect of ethanolic extracts of *S. canadensis* against *B. campestris.*

Leave numbers

Number of leaves developed of treated *B. campestris* was highly influenced by crushed dry leaves of the tested plants (Fig. 1D). For treatment using *M. azedarach* leaves, a significant reduction was recorded; however, this reduction was less than those treated with *P. nigra* leaves. The reduction value was proportional to its weight and recorded the highest at 300g of either studied plants (5.83 and 4.30 for *M. azedarach* and *P. nigra*, respectively) in comparison to control (7.83).

Internode numbers and its length

The amount of crushed dry leaf of the examined plants had a significant impact on the number of internodes and their length (Fig. 2A and B). For internode number, it showed a reduction when either *M. azedarach* or *P. nigra* leaves were used. Similar results obtained, as other growth parameters, in which increasing the weight of the dry leaves less number of internode was recorded compared to control (5.2 and 6.24 for 300g dry leaves and control plants, respectively).
In the case of treatment with *P. nigra* leaves, highest reduction of internode numbers were recorded in 300g (3.83) compared to control (6.73).

In general, the inhibitory impact of dry leaves became more severe as the dose was increased. For internode length, an increase was observed for *B. campestris* when treated with either crushed dry leaves of *M. azedarach* and *P. nigra* enhanced (Fig. 2B).

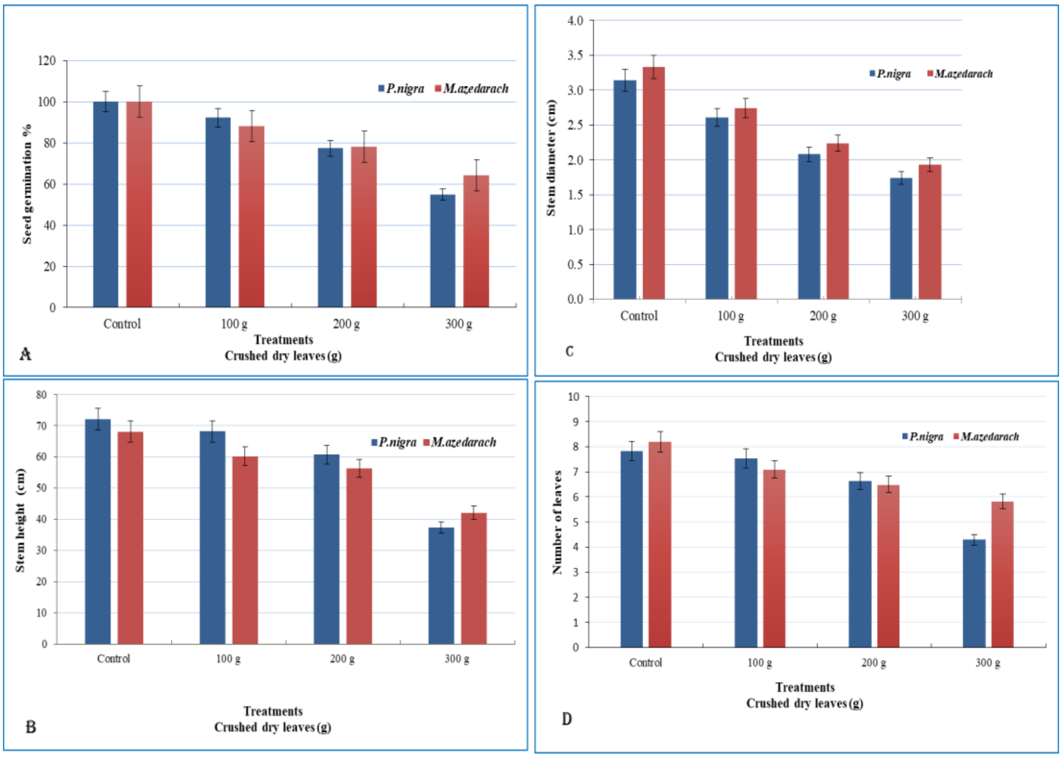

Figure (1): Allelopathic effect of *M. azedarach* and *P. nigra* leaves e on growth parameters of *B. campestris.* A, seed germination %; B, stem height; C, stem diameter and D, numbers of leaves per plant.

P. nigra leaves was more effective than *M. azedarach.* The highest effect was observed in 300g which recorded 14.20cm compared to control (10.5 cm).

Flower number

The number of flowers decreased as the weight of dry leaves of tested plants increased (Fig. 2C). In the case of *M. azedarach* leaves, the flower numbers decreased dramatically at 300 g and recorded 41.06% reduction compared to control plants. The reduction in flower number decreased as amount of added leave decrease (19.23 and 5.38 for 200 and 100 g of dry leaves, respectively). Meanwhile, *P. nigra* treatment showed less reduction (38.09 %) compared to control for the highest amount of dry leaves used (Fig. 2C).

Inflorescence size

The size of *B. campestris* inflorescence was highly influenced by the dry leaves of the examined plants (Fig. 2D). Although *M. azedarach* leaves had a stronger inhibitory effect than *P. nigra* leaves, there was no statistically significant difference. This inhibitory effect was recorded with *P. nigra* bark sown in fields which had adversely affected regarding *Zea maize* growth and ultimately resulting in lower yield (Khattak *et al*., 2016).

Fresh and dry weight of stem

Data obtained for the effect of crushed dry leaves of both *M. azedarach* and *P. nigra* revealed that with increasing plant treatment concentration stem fresh and dry weight was inhibited (Table 1). In the case of *M azedarach*, the inhibitory effect was more expressed (Table 1). Grinded leaves (300g) recorded significant ($p \leq 0.05$) repressive effect compared to control and *P.ngira* leaves (2.90; 4.24 and 3.09 g, respectively). Meanwhile, dry leaves, at all doses used, of both tested plants had greater influence on the measured parameters (Table 1). However, increasing the weight dose of the leaves had a corresponding harmful allopathic impact. In general, the allelochemicals present in these plants supressed all growth parameters of surrounding cultivated plants (Khattak *et al*., 2016).

Weight of fixed number of seed

According to the findings (Table 1), the dry seed weight of 1000 fixed number was negatively affected as the treatments increased. The weight of 1000 *B. campestris* seeds decreased as the dose of *M. azedarach* dry leaves treatments increased. Meanwhile, *P. nigra* treatments had a lower effect on all assessed parameters but were significantly higher than *M. azedarach* for 1000g seed weight.

Lab experiment

Effects of P. nigra leave-extract on B. campestris

Seed germination percentage

According to the results of laboratory studies (Table 2), increasing concentrations of *P.nigra* fresh and dried leaves extract inhibited the germination percentage of *B. campestris*. At 72 hours of soaking, each concentration of both fresh and dry leaves had a negative effect. Germinating seeds suppression was maximum in 20g concentration at 72hr soaking period of fresh leaves (20%) and dried leaves (30%) as compared to control (100%). Although, germination % was also reduced at tested concentrations of 15g and 10g, but was significantly ($p \leq 0.05$) less.

Plumule length of B. campestris

Table (2) shows that increasing the content of *P. nigra* fresh and dry leaves in plant extract inhibited growth as measured by plumule length. After 72 hours the substantial inhibitory impact was more pronounced, at 20g of leaf soak.

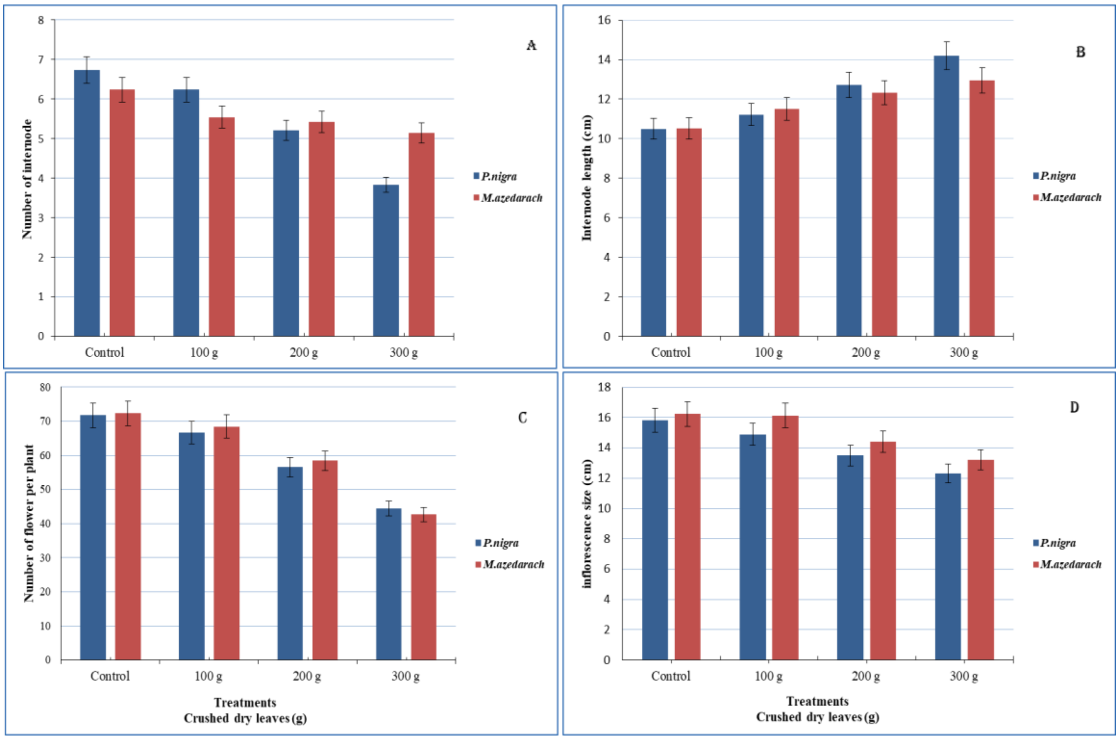

Figure (2): Allelopathic effects of varied *P.nigra* and *M. azedarach* dry leaf - doses on assessed parameters of *B. campestris*. A, number of internode; B, Internode length; C, Number of flowers per plant; and D, inflorescence size.

Table (1): Allelopathic effect of *P. nigra* and *M. azedarach* dry leaves, at different studied doses (g), on stem fresh and dry weight and weight of 1000-seeds.

Treatments (g Crushed dry leaves)	Stem				1000 seed weight (g)	
	Fresh weight		Dry weight			
	1	2	1	2	1	2
Control	4.51 ±0.10 [a]	4.24 ±0.30 [a]	3.10 ±0.07[a]	3.30 ±0.10[a]	3.94 ±0.20[a]	4.03 ±0.11[a]
100	3.52 ±0.05 [b]	3.40 ±0.73 [b]	2.54 ±0.09	2.90 ±0.09[b]	3.85 ±0.50[a]	3.71 ±0.09[b]
200	3.26 ±0.08 [b]	3.40 ±0.77 [b]	2.34 ±0.08	2.02 ±0.50[bc]	3.33 ±0.32[b]	3.54 ±0.21[b]
300	3.04 ±0.08 [c]	2.90 ±0.32 [c]	2.03 ±0.04	1.62 ±0.10[c]	2.93 ±0.12[c]	3.22 ±0.50[c]

1, *P. nigra;* 2, *M. azedarach;* data are in mean±SE; data with different letter, per each column, are significantly different at $p \leq 0.05$ based on Duncan's Multiple Range Test.

Radical length of B. campestris seedlings

The results (Table 2) showed that increasing the amount of *P.nigra* fresh and dry-leaves weight for extract preparation resulted in suppression of radicle growth as measured by radical length. When compared to the control, the suppression was greatly reduced for using dry and fresh (84.6 and68.7%, respectively). The maximum inhibitory effect was obtained at 72hr soaking period of both fresh and dry leaves extract utilising 20g of plant-leaves either dry or fresh leaves. When dry leaves extract was applied, radicle length was increased (Table 2).

DISCUSSION

According to our findings, *P. nigra* and *M. azedarach* leaf extracts have negative allelopathic effects on *B. campestris* in terms of Germination percentage, leaves number, leaf length, stem height, stem diameter, numbers of internodes, internodal length, numbers of flowers, size of inflorescence, stem fresh weight, dry weight, and 1000 grains-weight. Except for internodal length, all metrics were shown to be adversely influenced (all parameters decreased with an increase in concentration while internodal length increased). The inhibitory effects of 300g treatments were greater than those of 200g and 100g treatments. These data are in agreement with does done by Lava *et al*., (2015).

Negative allelopathic effects of aqueous extracts of both fresh and dried leaves of *P. nigra* (10, 15 and 20g/100ml) on germination %, plumule, and radicle length of *B. campestris* were also observed in laboratory trials. The greatest inhibition was reported after 72 hours of leaf soaking at a concentration of 20g. These findings are comparable to those of Kavitha, (2015), who discovered that extracts of *C. rotundus* had inhibiting effect on seed germination, seedling growth, and biomass production of *Brassica* species.

In another studies done by Romel *et al.* (2007) and Kato-Noguchi (2021), also showed allelopathic effects of *Lantana camera* leaves on the seed germination and growth of *B. juncea*, *C. sativa,* and *Raphanus.* In parallel, Lava and his colleagues (2015) demonstrated the phytotoxic effects of *M. azedarach* L. dried leaves and their seed extracts on the growth parameters of *Cicer arietinum* L. (chickpea). This outcome is consistent with our results. Mean-while, increasing the concentration of the treatments had a considerable inhibitory effect.

Similarly, the phytotoxic effects of *Ziziphus nummularia* (Rhamnaceae) leaf, bark, and fruit on *Vigna radiata* and *Brassica campestris* seeds were consistent with our findings, where plumule and radical growth were significantly retarded by an aqueous extract of bark at all concentrations and soaking times. Singh *et al.* (2009) found phytotoxicity of *Parthenium hysteronnphorus* residues on the growth parameters of three tested *Brassica* species (*B. campestris, B. oleracea* and *B. rapa*). In a trial to explore the inhibitory mechanism (Phuwiwat *et al*, 2012; Hussain and Abbas, 2021) these researches revealed allelopathic effect caused by the release of water-soluble phenolic into the soil. *M. azedarach* leaves significantly suppressed the shoot dry weight of mung bean, which was analogous to our findings (Shapla *et al.*, 2011).

In addition, Allelopathic effects of *M. azedarach* L. leaf litter and leaf aqueous extracts on germination, growth, and yield of *Vigna mungo* L. (black gramme) and *Cicer arietinum* L. were reported by Kumar *et al.* (2017), in which both leaf aqueous extract and leaf litter supressed the germination, initial growth, and biomass of black gramme and chickpea.

CONCLUSION

The current study shown that the allelopathic effect of either *P. nigra* or *M. azedarach* fallen leaves on growing crops, including the investigated plant *B. campestri*s L. Meanwhile, these leaves of either *P. nigra* or *M. azedarach* may contain allelochemical substances that inhibit seed germination as well as growth of *Brassica campestris* L. represented by inhibition of radical and plumule growth. In the meantime, the aqueous extract of these leaves on a lab level also inhabits *B. campestris* L seed germination due to release of these substances. Therefore, leaves of these particular trees should be removed from fields before sowing any crops and in particular *Brassica campestris* L. Also, it is recommended to avoid cultivation of *Brassica campestris* L in the area where *M. azedarach* and *P. nigra* are grown.

Table (2): Allopathic effect of dry and fresh leaves of *P.niger,* at different weight 10, 15 and 20g soaked in 100 ml distilled water, on seed germination(%) and growth parameters of germinated seeds represented by plumule and radical length of *Brassica compestris*.

Treatments (g)	Seed germination %		Measured growth parameters			
			Plumule length (cm)		Radical length (cm)	
	1	2	1	2	1	2
Control	100.0	100.0 ^{a}	5.50 ± 0.08 ^{a}	4.47 ±0.11 ^{a}	6.32 ±0.21	5.43 ±0.14 ^{a}
10	83.8	90.0 ^{b}	2.53 ±0.16 ^{b}	3.12 ±0.06 ^{b}	2.05 ±0.09	3.53 ±0.25 ^{b}
15	65.0	78.8 ^{c}	1.15 ±0.11 ^{c}	2.09 ±0.19 ^{c}	0.96 ±0.02	2.36 ±0.09 ^{c}
20	85.3	66.3 ^{d}	0.39 ±0.28 ^{d}	1.23 ±0.16 ^{d}	0.41 ±0.02	1.70 ±0.11 ^{d}

1, dry leave*; 2,* fresh leaves*;* data are in mean±SE; data with different letter, per each column, are significantly different at $p \leq 0.05$ based on Duncan's Multiple Range Test.

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