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Comparative status of foliar pigments, nutrients, polyphenol, flavonoids and antioxidant activity of *Vitex negundo* L. in three different growing seasons

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Abstract
Comparative status of foliar photosynthetic pigments, nutrients, polyphenol, flavonoids, and antioxidant activities of *Vitex negundo* L. were studied in three different growing seasons within a generation. An experiment was managed during February, 2019 to January, 2020 at the Botanical Garden, University of Chittagong. Maximum foliar photosynthetic pigments, nutrients were assessed in monsoon (June-August) and minimum in late monsoon (September-November) respectively. Meanwhile the highest flavonoids content was observed in the leaf extract of early monsoon (March-May) and the lowest in late monsoon (September-November) respectively. Moreover the maximum polyphenol and antioxidant activity were estimated in the leaf extract of late monsoon (September-November) and the lowest in early monsoon (March-May) respectively. Present study concludes that monsoon (June-August) is competent for efficient photosynthesis as well as nutrient assemblage in *Vitex* and early monsoon (March-May) is for flavonoid synthesis and late monsoon (September-November) is suitable for collecting leaves of *Vitex* to be acquired highest antioxidant activity and polyphenol content for medicinal use.

Keywords: Pigments, comparative studies, *Vitex*, nutrients, antioxidant, polyphenol, flavonoids

Introduction
Vitex negundo L. is an important medicinal, aromatic, deciduous, woody shrub to small tree belongs to the family Verbenaceae, locally familiar as 'Nirgundi' [1-3]. The plant is upright, willowy small tree about 2-4m in height, tri to penta foliate, lanceolate leaf, 4-10cm long, palmately arrangement, with quadrangular shoot and recorded to obtain Bangladesh, India, Afghanistan Sri Lanka, Pakistan Malaysia, Thailand, Madagascar and eastern Africa [4, 5]. It is grown as a crop in parts of Asia, North America, Europe and West Indies and also source of timber [6]. It is one of the most powerful medicinal plant and has distinct pharmacological activity *viz.* anti-diabetic [7, 8], anticancer [9, 10], anti-oxidant [11, 12], anti-inflammatory [13, 14], antimicrobial [15, 16], anti-osteoporotic [17, 18], anti-cataract [19], hepatoprotective [20, 21], antiandrogenic [22], insecticidal [23, 24] and anti-venom [25]. A total of 120 different compounds *viz.* steroids, terpenoids, flavonoids and lignans isolated from the different parts of plant [26].
In literature survey that no particular research has yet been done on the seasonal effects of active components in *Vitex* plant in regard to seasons and habitat in Bangladesh. The present research was assume to evaluate the comparative interpretation on foliar photosynthetic pigments, nutrients, polyphenols, flavonoids, and antioxidant status of *Vitex negundo* L. in three different seasons.

Materials and Methods
The experimental leaf samples of *Vitex negundo* L. were collected in three different seasons (EM: Early monsoon – March, April and May; M: Monsoon-June, July and August; LM: Late Monsoon- September, October and November) from the Botanical garden, University of Chittagong. Leaves were dried in the laboratory under current air at room temperature (28 ^{0}C) and then after twenty four hours the materials were put in to the oven maintained at 60 ^{0}C for 48 hours. These were then weighed in an electric balance and ground to pass through 0.2 mm sieve and preserved in airtight plastic vial for analysis.
Foliar photosynthetic pigments were estimated by Wettstein method [27]. For this purpose fresh leaves were collected from the plants. Foliar nutrients (*viz.* N, P and K) were extracted with sulfuric-peroxide (H_2SO_4+H_2O_2) digestion mixture and evaluated by standard method [28]. Flavonoids were determined using an UV-visible spectrophotometer (Shimadzu UV-160A PC, Shimadzu Corporation, Kyoto, Japan) method [29]. The polyphenol estimated based on Roberts [30] with modification from the recent work of some researchers [31, 32]. The radical scavenging activity of the leaf extracts was evaluated by the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical using a modified method [33]. The assay is based on the quantification of the scavenging ability of antioxidants towards the stable DPPH radical [34]. There were three replications for each set of experiment. Experiments were studied on complete randomize design. Statistical analyses were done pursuant to MS excel.

Results & Discussion
The results (Table-1, Fig.1) expose that the foliar photosynthetic pigments diverse with the change of seasons. Chlorophyll-a, Chlorophyll-b, Carotenoids, total chlorophyll and total pigments varied from 1.415 mgg^{-1} FW (Monsoon) to 1.181 mgg^{-1} FW (Late Monsoon); 1.338 mgg^{-1} FW (Monsoon) to 0.759 mgg^{-1} FW (Late Monsoon); 0.814 mgg^{-1} FW (Monsoon) to 0.636 mgg^{-1} FW (Late Monsoon); 2.75 mgg^{-1} FW (Monsoon) to 1.75 mgg^{-1} FW(Late Monsoon); and 3.57 mgg^{-1} FW (Monsoon) to 2.38 mgg^{-1} FW (Late Monsoon) respectively and showed the following sequence

as M>EM>LM. ANOVA of foliar total pigments showed significant value (P<0.01) with seasons (Table-2). Charu and Vandana (2010) [35] showed highest chlorophyll-a content in monsoon and lowest in late monsoon in *Jatropa curcas* and *Acacia nilotica*. Prajapati and Tripathi (2008) [36] enumerated highest total chlorophyll content in monsoon and lowest in late monsoon in the leaf of *Ficus religiosa*, *Psidium guajava*, *Mangifera indica* and *Dalbergia sissoo*. In case of plucked shoots of clonal agrotypes of tea and kalomegh, photosynthetic total pigments were found to be changed with plucking seasons and highest value was achieved in monsoon [37, 38] which are analogous to this finding.

The result of foliar nutrients *viz.* Nitrogen, Phosphorus and Potassium are presented in Fig. 2. Foliar Nitrogen, Phosphorus and Potassium contents fluctuated from 4.39% (Monsoon) to 3.14% (Late Monsoon); 0.49% (Monsoon) to 0.34% (Late Monsoon); and 3.49% (Monsoon) to 2.40% (Late Monsoon); respectively and showed the following trend as M>EM>LM. ANOVA of foliar N, P and K appeared significant value (P<0.01) with seasons (Table-2). Patarapanich *et al*. (2007) [39] and Uddin *et al*. (2019-20) [40] observed that the foliar nutrients of *Andrographis paniculata* and *Gynura procumbens* exchanged with seasons as well as growing conditions. These observations also bear a similarity to the findings of the present study.

Table 1: Change of foliar pigments of *Vitex negundo* L. in three different growing seasons.

Seasons	Pigments, mg/g FW				Ratio of pigments		
	Chl-a	Chl-b	Car	Total Chl	Chl-a/Chl-b	Chl-a/Car	Chl/Car
EM	1.181±0.057	0.886±0.087	0.669±0.058	2.07±0.13	1.339±0.11	1.77±0.06	3.099±0.16
M	1.415±0.046	1.338±0.044	0.814±0.055	2.75±0.09	1.057±0.10	1.75±0.17	3.399±0.33
LM	0.985±0.018	0.759±0.019	0.6363±0.038	1.74±0.06	1.298±0.11	1.294±0.41	2.747±0.14
F -value	60.69*	64.81*	8.17**	74.67*	13.77**	2.56**	4.88*

Legend: Chl= Chlorophyll; Car= Carotenoids; *= Significant at 1% level; **= Significant at 5% level; FW= Fresh weight; EM=Early Monsoon; M=Monsoon; LM=Late Monsoon.

Table 2: Analyses of variance of total pigments, nitrogen, phosphorus, potassium, flavonoids, polyphenol and antioxidant content in leaf of *Vitex negundo* L. in three different seasons.

Source of variance	Degree of freedom	F-values						
		N	P	K	Total pigments	Flavonoids	Polyphenol	Antioxidant
Seasons	2	18.8*	16.8*	16.9*	140.63*	37.8*	27.4*	46.9*
Error	6	-	-	-	-	-	-	-

Legend: * denotes significant at 1% level.

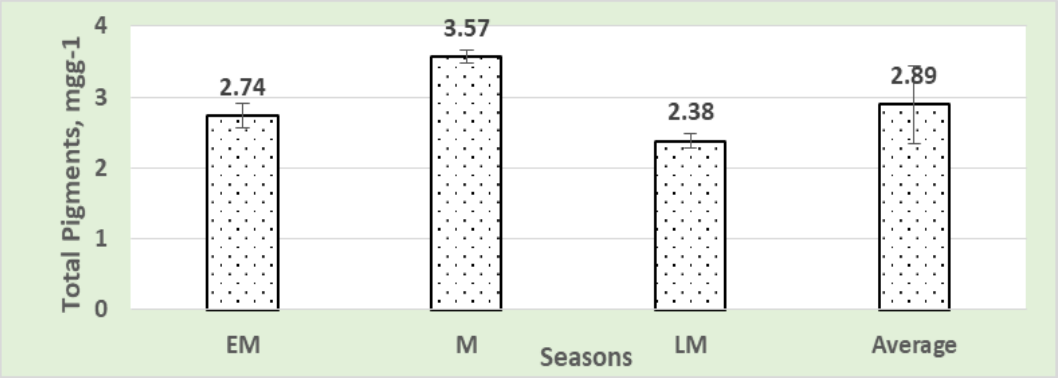

Fig 1: Change of total foliar pigments of *Vitex negundo* L. in three different growing seasons.

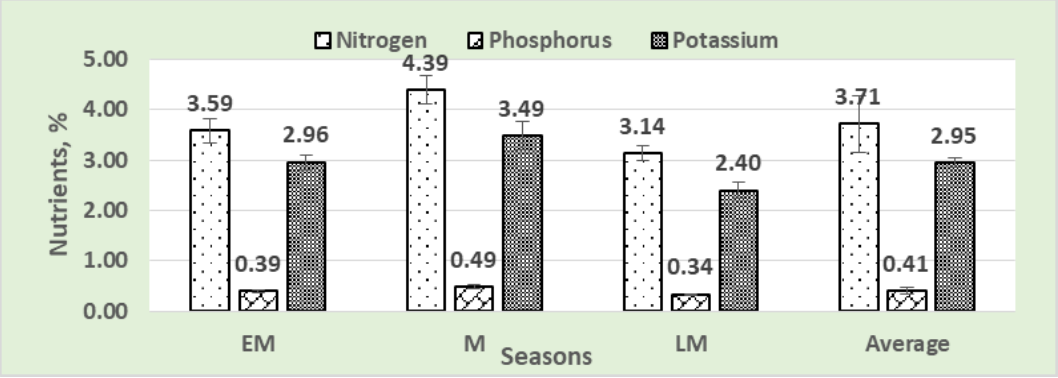

Fig 2: Change of foliar nutrient status of *Vitex negundo* L. in three different growing seasons.

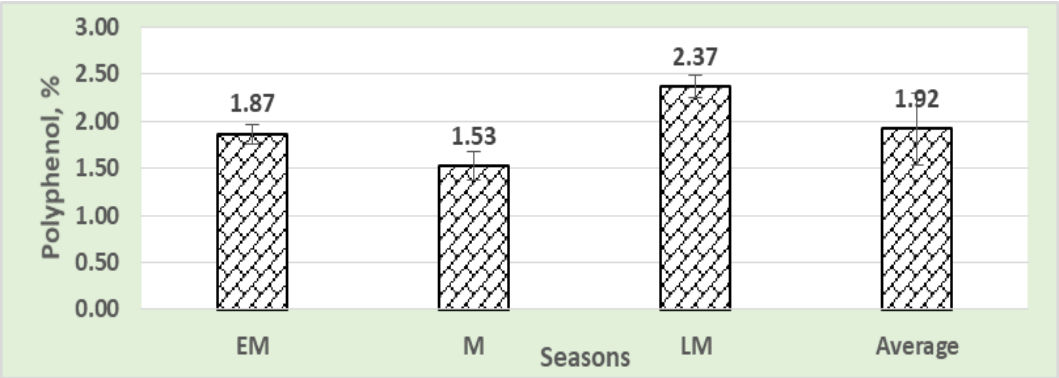

Fig 3: Change of foliar polyphenol status in the leaves of *Vitex negundo* L. in three different growing seasons.

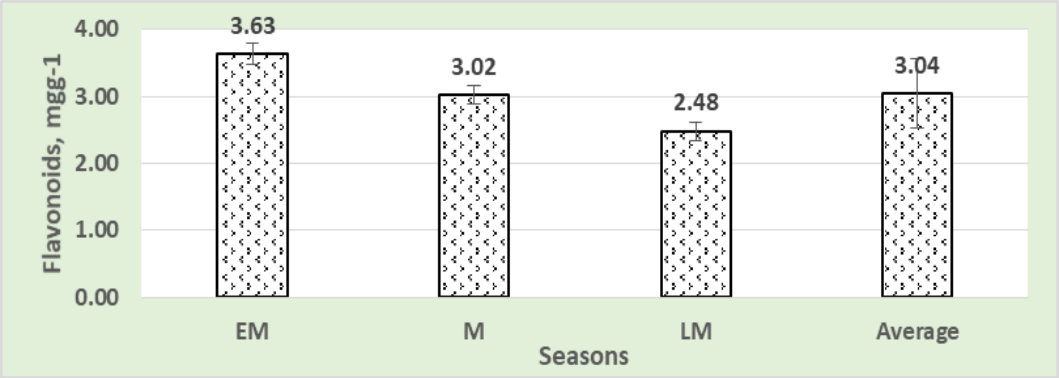

Fig 4: Change of foliar flavonoids content in the leaves of *Vitex negundo* L. in three different growing seasons.

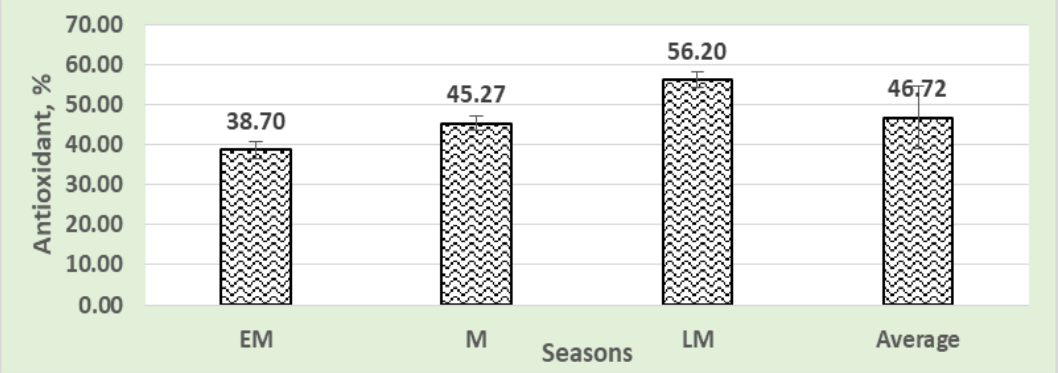

Fig 5: Change of foliar antioxidant activity of *Vitex negundo* L. in three different growing seasons.

The results of foliar polyphenol contents are displayed in Fig. 3. Foliar polyphenol contents changed from 2.37% (Late Monsoon) to 1.53% (Monsoon) and showed the following trend as LM>EM>M. ANOVA of foliar polyphenol exhibited significant value (P<0.01) with seasons (Table-2). Polyphenol content was highest in late monsoon and lowest in monsoon. Wahba *et al.*, 2017 [41] in *Cynara cardunculus* observed highest polyphenol content in early monsoon (May) than monsoon (June). A possible cause of highest polyphenol content in late monsoon and lowest in monsoon due to the enzyme polyphenol oxidase (PPO) shows the maximum activity in monsoon (rainy season) and low activity in late monsoon suggested by Thakur and Kapila (2017) [42]. In liverworts Thakur & Kapila (2017) [42] evaluated highest phenolic content in winter (late monsoon) and lowest in rainy season (monsoon). Liao *et al.* (2018) [43] measured highest phenolic content in winter (late monsoon) in the shoot extract of *Oxalis corymbosa* which are similar to this finding.

The results of foliar flavonoids contents are shown in Fig. 4. Foliar flavonoids contents ranged from 3.63 $mgg^{-1}{}_{DW}$ (Early Monsoon) to 2.48 $mgg^{-1}{}_{DW}$ (Late Monsoon) and showed the following progression as EM>M>LM. ANOVA of foliar flavonoids showed significant value (P<0.01) with seasons (Table-2). Zhu *et al.* (2013) [44] noticed that the maximum content of total flavonoids of *Vaccinium ashei* leaves was achieved in the month of May (Early Monsoon). Cezarotto *et al.* (2017) [45] estimated that the maximum content of total flavonoids of *Vaccinium ashei* leaves was acquired in the month of April (Early Monsoon) which substantiate with this finding.

Foliar antioxidant activity are exposed in Fig. 5. Foliar antioxidant activity altered from 56.20% (Late Monsoon) to 38.70% (Early Monsoon) and showed the following succession as LM>M>EM. ANOVA of foliar antioxidant activity appeared significant value (P<0.01) with seasons (Table-2). Zhu *et al.*, 2013 [44] exhibited that the rabbiteye blueberry leaves from November had the maximum antioxidant capacity and Rekha *et al.*, 2015 [46] and Cao *et*

al., 2019 [47] also proclaimed that one of the highest antioxidant activity was achieved in the month of November (Late Monsoon) in the leaf extracts of *Ligularia fischeri* and *Cyclocarya paliurus* respectively. These reports are reconcilable with the present experiment.

Conclusion

The present study accomplishes that monsoon (June-August) is competent for efficient photosynthesis as well as nutrient assortment in *Vitex* and early monsoon (March-May) is suitable for flavonoid synthesis. Contemplating the number of all studied elements late monsoon (September-November) is the proper time for harvesting the leaves of *Vitex negundo* to be achieved highest polyphenol content and antioxidant activity for medicinal use.

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