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Title: Ecomorphometric Factors Account To Deviations In The Amount of Vasicinein*adhatodavasica*nees

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Abstract:

Vasicine; known for its pharmacological functional annotations, is a pyrrolo-quinazoline alkaloid present in *Adhatodavasica*Nees. In this study we ascertain the relationship between vasicine and ecomorphometriccomponents of the plant. Our findings suggest a considerable influence of qualitative factors like purple streaks of corolla on the metabolite accumulation. Fading or lack of purple marks on corolla ispositively correlated to soil organic carbon, veins per leaf, leaf length and vasicine. Further a prominent negatively linear relationship is evident for pH and vasicine content. With a pronounced non-linear trend, the content of vasicineis higher atmiddle values. In this paper our results revealed the importance of optimum conditions for metabolite production and the role of eco-morphological factors on vasicine.

Key words:vasicine; *Adhatodavasica*; ecomorphological characters; chemotypes

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

Introduction:

*Adhatodavasica*Neesof the family Acanthaceaeis an endemic plant of tropical Asian countries[1]. It contains active secondary metabolites that are invaluable in traditional and modern medicine. With decades of applications in medicine,vasicine still holds promise for un-ascribed diseases.Harvesting improved quantities of vasicine and identifying right chemotypesare of considerable importance in this field. For the identification of right chemotypes, understanding eco-morphological parameters and their relationship with the metabolite accumulation finds research attention. Environmental and edaphic conditions shape the chemistry and build-up of active metabolites in plants.Additionally, the past studies leave prominent gaps in evaluating independent variables accounting to metabolite accumulation in *A. vasica*.For example, according to Raja *et al.*,[2]representatives of *Adhatodavasica*from different places of Southern India exhibited significant differences in chemical and pharmacognostic characters. But the parameters are not correlated with vasicine content. Some reports[3] suggest the profile of the active metabolites of *A. vasica* with changingseasons,while others report scattered information without drawing any conclusions based on correlation [4,5].Hence in this study, we have made an attempt to understand the influence of independent ecomorphological variables on vasicineamong the accessions of *A. vasica*.

Materials and Methods:

Hand held GPS unit (Trimble make, Geo Explorer 6000) was deployed for recording the topological and geographical coordinates of different data points. Samples of *Adhatodavasica* were identified from 8topogeographical areas of Palakkad and Malappuram Districtsof Kerala ***(Table.1)***.

Table 1 Sample locations

Sample ID.	Name of the sample spot	Geographical coordinates	Herbarium Voucher No.
01	Kakkayur	elev 2.15 m, 10º39ˡ26.165ˡˡN; 76º39ˡ01.357ˡˡE	90874
02	Kanjikode	elev 25.25 m, 10º48ˡ08.079ˡˡN; 76º45ˡ02.902ˡˡE	90873
03	Kuzhalmannam	elev -19.89 m, 10º42ˡ06.004ˡˡN; 76º35ˡ31.082ˡˡE	90867
04	Kava, Malampuzaha Dam	elev 39.18 m, 10º49ˡ33.948ˡˡN; 76º44ˡ06.648ˡˡE	90879
05	Nelliampathi Plateau-1	elev 906.85 m, 10º32ˡ06.736ˡˡN; 76º41ˡ26.022ˡˡE	90869
06	Nelliampathi Plateau-2	elev 923.04 m, 10º32ˡ21.170ˡˡN; 76º41ˡ48.834ˡˡE	90870
07	Malapuram, Perinthalmanna	elev 21.97 m, 10º57ˡ46.114ˡˡN; 76º17ˡ21.424ˡˡE	90871
08	Wadakencherry	elev -27.16 m, 10º34ˡ03.347ˡˡN; 76º29ˡ31.569ˡˡE	90876

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

Samples were collected during the field visits in the flowering season (February) of the plants.Soil samples were collected from one foot depth from the soil surface. Soil samples were processed after shade drying and sieving through a 1mm mesh to get fine powder. Subsequently the processed soil samples were used for elemental analysis ***(Fig. 1)***. Air tight covers were used for sample storage until analysis. The plant floral axes were collected from each of the spots for developing herbarium specimens. The processed voucher specimens were submitted to Botanical Survey of India, Allahabadwhere complete taxonomic assessment was done and accession numbersare acquired ***(Table.1)***.

Fig.1. Soil sample preparation and storage

A sum total of 13 qualitative and 06 quantitative characters were identified for morphological characterization. Randomized samplingwasdeployed with07 plants from each location. Onsite collection and recording of morphological data was commissioned. Soil factors like total content of potassium[6,7], phosphorus [8] and organic carbon [9] were analysed across the samples following standard protocols. A pH meter was used for measuring the soil pH.Soil electrical conductivity (EC) was recordedusing a conductivity meter.

Results and Discussion:

Morphological and soil factors among the topogeographically distinct spots were characterized. Morphological analysis was ascertained byvaluingqualito-quantitativefactors. The deviations invisual morphologicalfactors are given in ***Table. 2*** and ***Table.3***. The purple markson the petals wereassociatedwith the amount of vasicine among thesamples. Lack of dark purple marks in some samples or faded presence insome samples***(Fig. 2a&b)***could berelated to relatively highervasicine; while on contraryevident purple marks on the corolla could be negatively related with amount ofvasicine in some samples***(Fig. 2c&d).***The in-between presence of the purple markscould be

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

negatively related to vasicineamong the data points.Plant height, veins on leaf, leaf length, leaf breadth, bract length and bract breadthdetails were collected for quantitative morphological analysis. These featuresshowed considerable difference across the data points as presented in ***Fig.3*.**The edaphicfactors were analyzed and the observed changes are given in ***Fig. 4***. Other than EC, rest of the parameters exhibited differences in distribution pattern across the study area. From our previously published report[10], the samples of Kanjikodeexhibited highest metabolite contentwhich is followed by Wadakencherrypresented in ***Fig.5*.** The vasicine from this data was correlated with morphological and soil factors.

Fig.2. Prominent purple streaks as determining factors of vasicine

Table 2 Qualitative vegetative factors

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

Sample ID	Habit	Leaf shape	Leaf tip	Mature leaf texture	Leaf margin	Leaf colour	Stem colour
1	Dense	Elliptic lanceolate	Acute	Thin, glabrous	Entire	Light green	Greenish brown
2	Dense	Elliptic lanceolate	Acute	Thin, glabrous	Entire	Light green	Greenish brown
3	Highly dense	Elliptic lanceolate, broad	Acute	Thick	Entire	Dark green	Greenish cement
4	Highly dense	Elliptic lanceolate	Acute	Thin	Entire	Light green	Pale greenish brown
5	**Huge shrubs**	Elliptic lanceolate, broad	Acute	Thick	Entire	Deep dark green	Greenish cement
6	**Huge shrubs**	Elliptic lanceolate, broad	Acute	Thick	Entire	Deep dark green	Greenish cement
7	Dense	Elliptic lanceolate	Acute	Thick, glabrous	Entire	Dark green	Brownish cement
8	Highly dense	Elliptic lanceolate	Acute	Moderate	Entire	Light green	Pale greenish brown

Table 3 Qualitative floral factors

Sample ID	Inflorescence	Calyx texture	Calyx type	Corolla colour	Corolla type	Corolla pubescence
1	Axillary spike	Pubescent	Oblong	White with prominent purple streaks	Bilipped	Yes
2	Axillary spike	Pubescent	Oblong	White, devoid of purple streaks	Bilipped	Yes
3	Axillary spike	Pubescent	Lanceolate	White with prominent purple streaks	Bilipped	Yes
4	Axillary spike	Pubescent	Lanceolate	White with faint purple streaks	Bilipped	Yes
5	Axillary spike	Pubescent	Oblong	White with prominent purple streaks	Bilipped	Yes
6	Axillary spike	Pubescent	Oblong	White with prominent purple streaks	Bilipped	Yes
7	Axillary spike	Pubescent	Oblong	White with moderate purple streaks	Bilipped	Yes
8	Axillary spike	Pubescent	Oblong	White with faint purple streaks	Bilipped	Yes

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

Fig. 3.Characterization data of morphological parameters

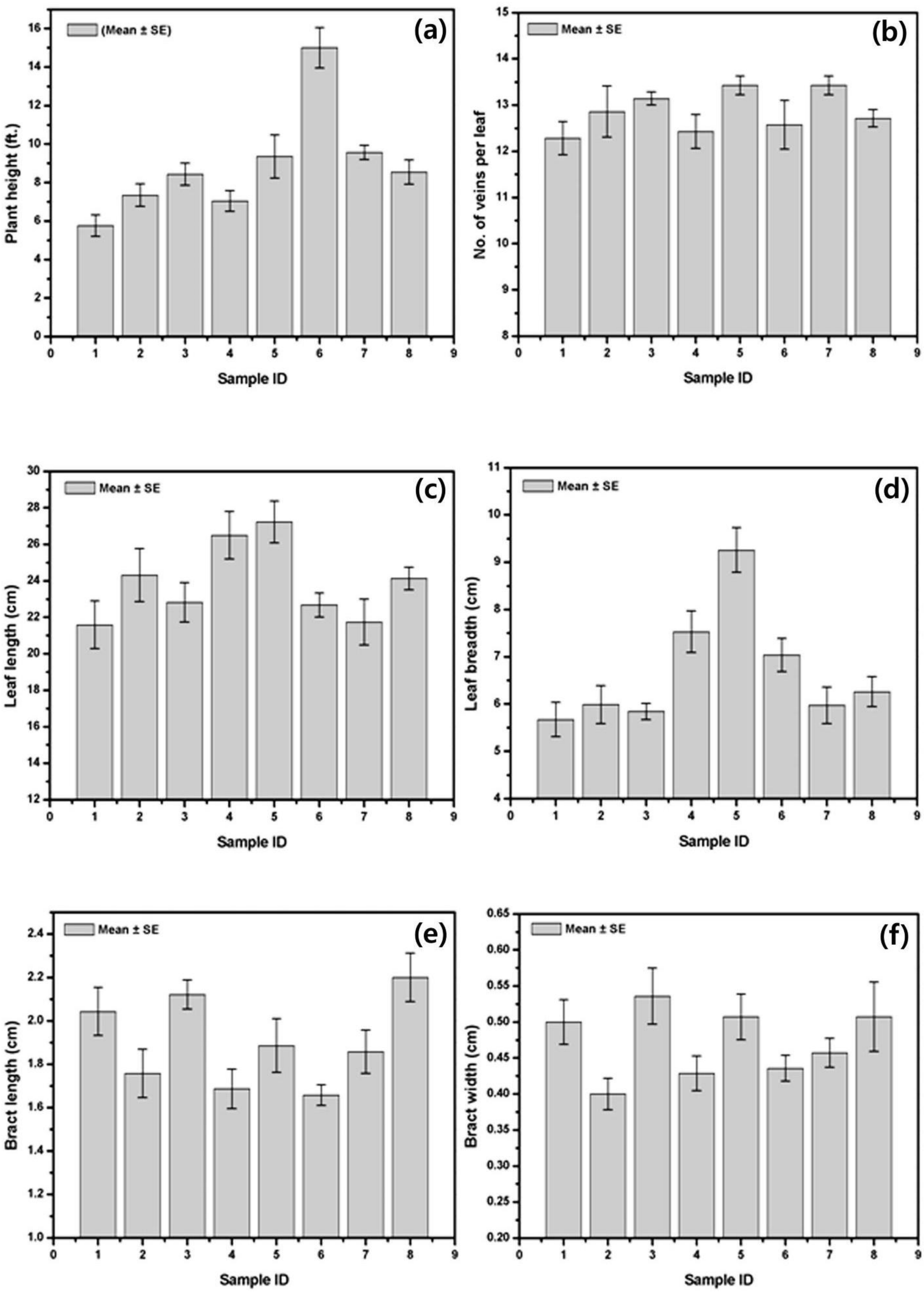


Fig. 4.Compositional characterization of edaphic parameters

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

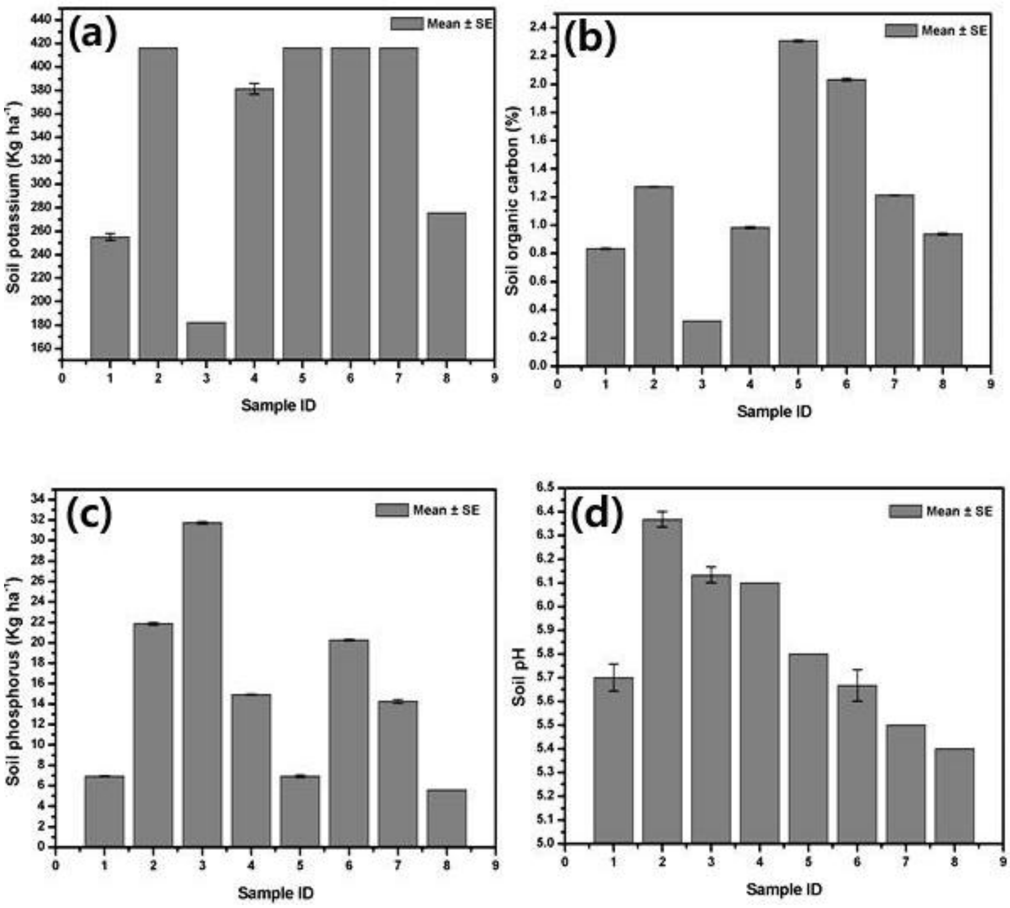
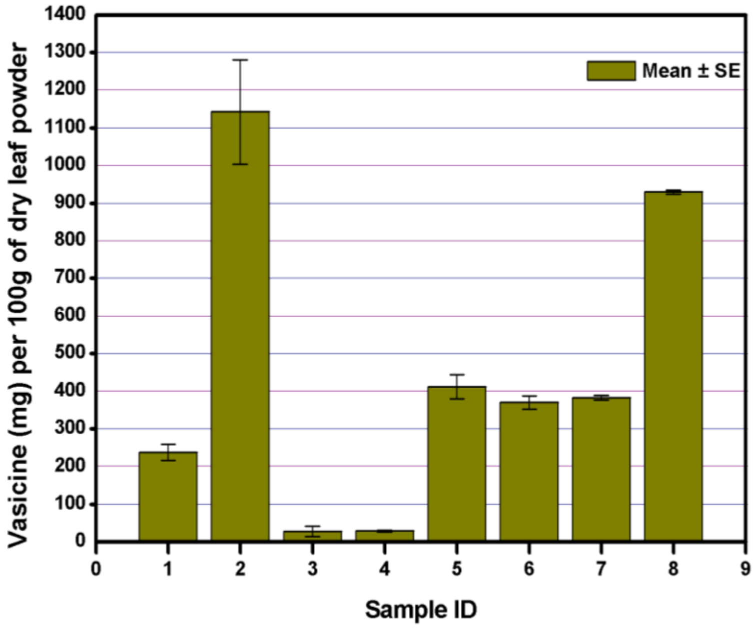


Fig. 5.Vasicine levels of *A. vasica*plants of the data points based onLC-MS/MS quantification



(Reproduced from our previous report -SaiMurali et.al., 2016) [10]

Apparent correlation between vasicine (dependent variable) andmorpho-edaphic factors(independent variables) is noticed. With the insights acquired from scatter plot analysis, evident non- linear, inverted "V" pattern between vasicine andindependent parameters was understood. Optimum No. of veins on leaf lamina was estimated as 13 (***Fig. 6***), length of the leaf needs to be24 cm (***Fig. 7***), OC less than 0.5 and more than 2, metabolite content was found to be low (***Fig.8***), vasicine was found to have conspicuous negatively linear association with pH, however, ID-2 treaded away from this pattern (***Fig. 9***).

Interestingly there seems to be an evolving trend between few of the independent factors and vasicine despite the limited sample size (n=8). Pronounced negatively linear association was realizedwiththe pH of soil and vasicineacross datapoints. For remaining independent parameters;veins, leaf length, leaf width and OClevels, a prominent inverted "V" type non-lineartrend was witnessed with vasicine. Precisely, vasicinelevelsweremore at in-between values and taper off at other places. Based on the argument of Arnold *et. al.*,one of the prerequisites for the accumulation of active principles is carbon[11]. This is substantiated by positive non-linearassociation of OC with vasicine in our study. Optimum soil pH helps in the uptake of elements by plants[12]. The bioavailability of soil ingredients are important for core metabolic pathways and influence the active principles.

Fig. 6.Association analysis between morphological and metabolite parameters on a scatter plot

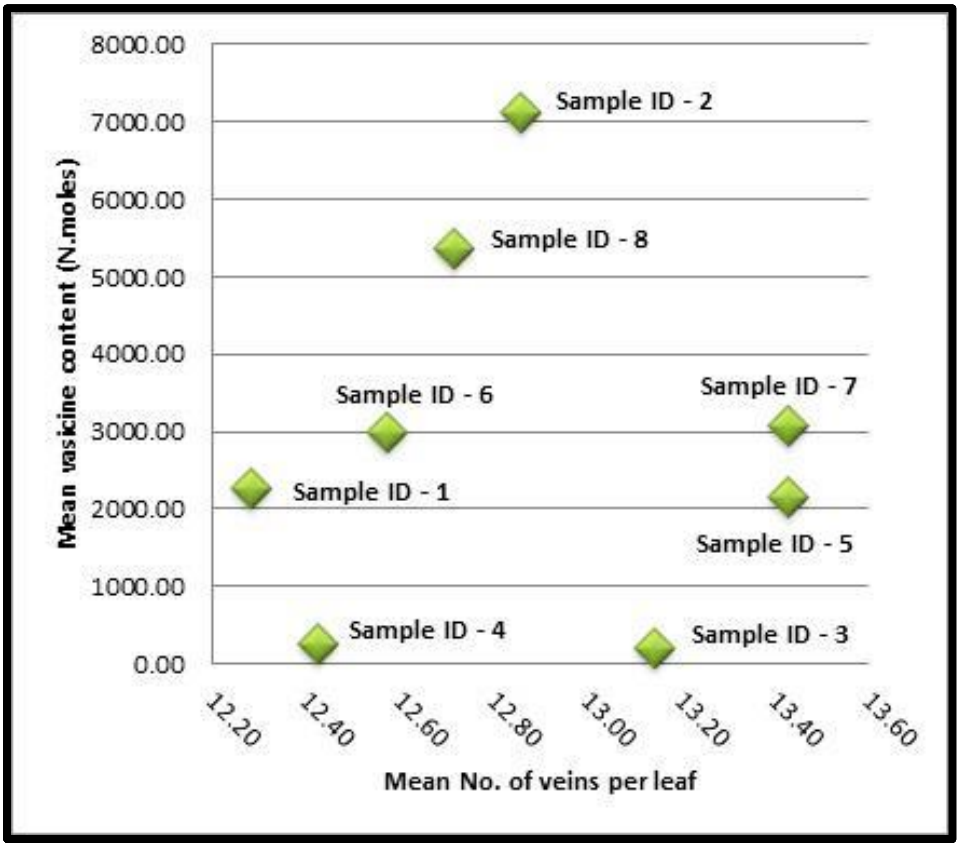


Fig.7. Association analysis between morphological and metabolite parameters on a scatter plot

ISSN: 0971-1260
Vol-22-Issue-16-August-2019

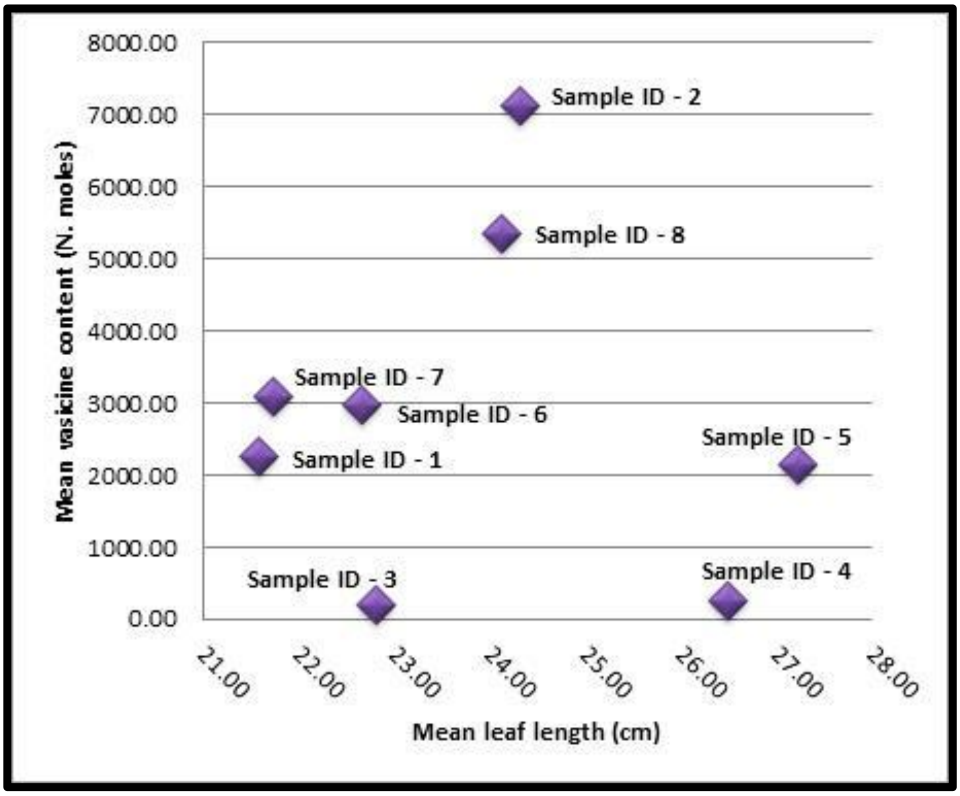


Fig. 8.Association analysis between edaphic and metabolite parameters on a scatter plot

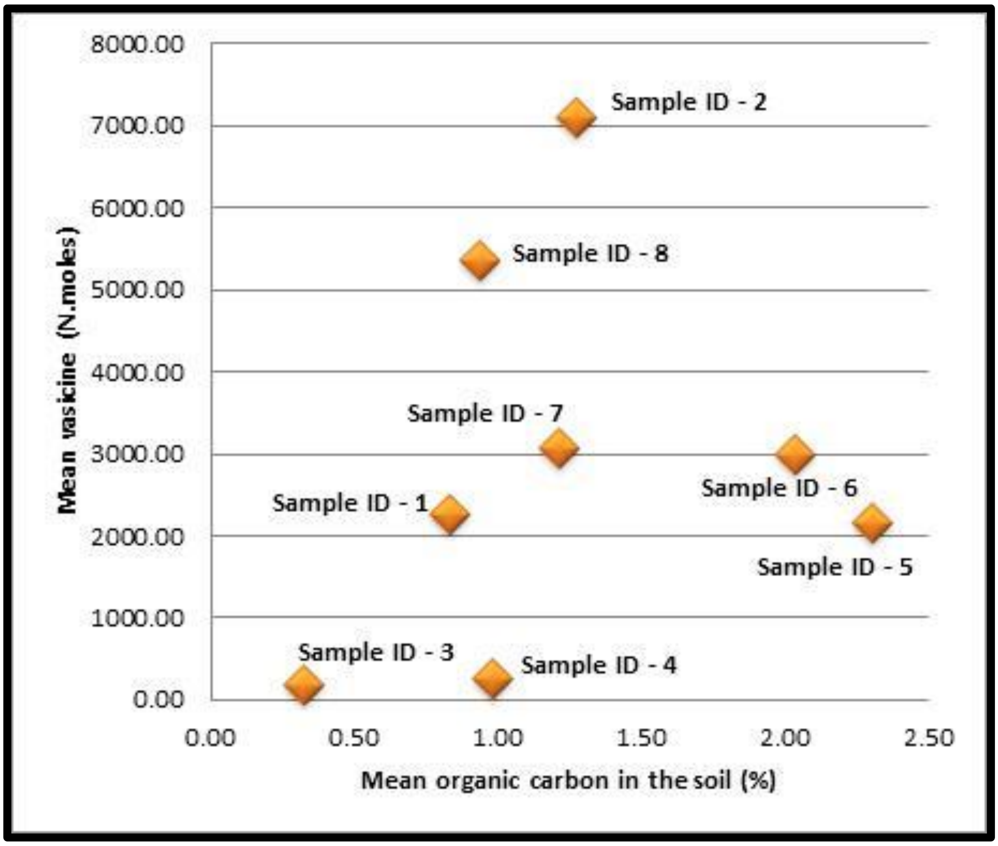
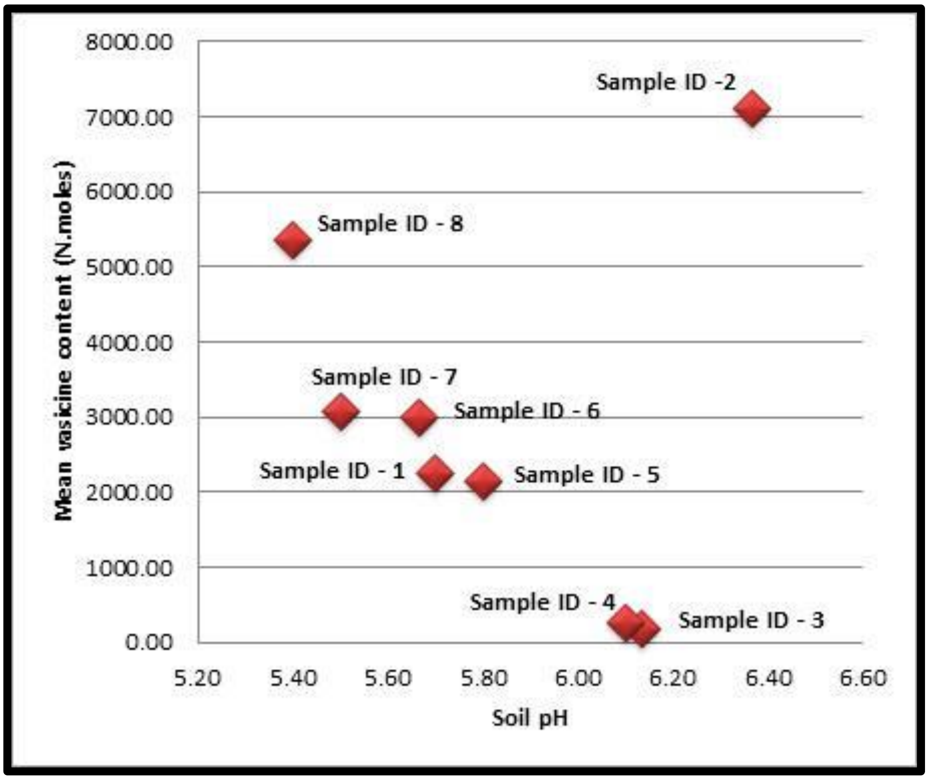


Fig. 9.Association analysis between edaphic and metabolite parameters on a scatter plot

ISSN: 0971-1260
Vol-22-Issue-16-August-2019



This is the first report on the dependence of vasicine on morphological and edaphic factors in *A. vasica*. To obtain robust statistical inferences and to understand non-linear relationships, future experiments may be planned with larger sample size. Such an extension work may help identify additional associations between morpho-edaphicfactors with vasicine.

Conclusions: Our study suggests considerable degree of influential relationship between ecomorphologial characteristics and quantity of vasicine in the plants. There is an evident correlation between the purple marks on the petals of corolla and vasicine. Deviation in the appearance ofpurple marks on petalsis linked with the non-linearpattern of relationship among certain morpho-edaphic parameters andvasicine. Pronouncednegatively linear correlation is observed between soil pH and vasicine. Additionally, non-linear relationship between vasicine andMorpho-edaphic parameters is noticed. In such cases, the amount of vasicine is higher at specificmiddle values. Furthermore, a larger study is imminent to ascertain comprehensive interactions.

Conflict of Interests: No conflict of interests is claimed by the authors

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