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Full Length Research Paper

Correlation between chemical profiles of black pepper (*Piper nigrum* L.) var. Panniyur-1 collected from different locations

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Black pepper (*Piper nigrum* L.) is known for its intrinsic quality. The volatile oil and pungent compounds are the two main components of black pepper. In the present study, dried berries of black pepper variety, Panniyur-1 were collected from eleven locations and subjected to variability studies for primary and secondary metabolites. A significant location wise variation was obtained for both primary and secondary metabolites. Variability was profound in essential oil, oleoresin, piperine, total phenol, crude fibre, starch, total fat and bulk density. A clear altitudinal variation was observed in β-caryophyllene and total phenol. These two constituents were low at high elevations (>500 mean sea level (MSL)) and high at plains. Similarly, monoterpenes like thujene, α-pinene, sabinene, limonene, α-phellandrene and linalool were relatively high at higher altitudes compared to plains. Total phenol, essential oil, piperine and oleoresin showed positive correlation with each other and also with crude fibre and total fat, but negatively correlated with bulk density and starch. Bulk density showed positive correlation with starch and negative correlation with all other constituents. There was a positive correlation between crude fibre and total fat. The study established that there was variability in aroma quality with respect to altitudes and it correlates differently with different constituents. This is the first report regarding altitudinal variation and correlation among metabolites of black pepper berries.

Key words: *Piper nigrum* L., essential oil, piperine, terpenes, caryophyllene, correlation.

INTRODUCTION

Black pepper of commerce is the dried mature fruits of the tropical, perennial climbing plant *Piper nigrum* L. which belongs to the family Piperaceae. Black pepper, the 'king of spices' contributes to a major share in the Indian spice scenario. Black pepper is found extensively in the evergreen forest of Western Ghats and nearby areas and is growing well in both plains and high altitudes (~ up to 1500 m mean sea level (MSL)). Black pepper provides physiological benefits and prevent chronic ailment in addition to the fundamental nutrition. It is an important healthy food owing to its antioxidant, antimicrobial, anticancer, anti-inflammatory and gastro protective effects (Butt et al., 2013). The volatile oil and pungent compounds are the two main components of black pepper. The alkaloid, piperine, is the major contribution to pungency whereas essential oil constituents like α- and β- pinene, limonene, myrcene, linalool, α-phellandrene, sabinene, β-caryophyllene, germacrene- D, etc., are the major aroma and flavor compounds of pepper (Jirovetz et al., 2002; Jagella and Grosch, 1999). Murthy and Bhattacharya (2008) reported that characteristic odour of black pepper is due to the volatile oil in the cells of pericarp. Variability in essential oil constituents was reported by several researchers. This can be attributed to the effect of cultivar, agro climatic variation, variation in the maturity of raw material, oil extraction

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method, etc (Zachariah and Parthasarathy, 2008). Kumoro et al. (2010) explained the differences in conventional hydrodistillation method and super critical fluid extraction method for essential oil extraction from black pepper. Andrade and Ferreira (2013) investigated the quality of black pepper extracts obtained by super critical fluid extraction (SFE) at different conditions of pressures and temperatures to evaluate yield behavior and anti oxidant activity. SFE gave fewer yields but more antioxidant activity as compared to conventional solvent extraction methods. Zachariah (1995) evaluated black pepper accessions collected from Indian Institute of Spices Research (IISR) Experimental Farm, Peruvannamuzhi, Kerala. Good variability was observed for both flavor and quality. Pinene content varied from 3.8 to 16.6%, sabinene from 2.2 from 33% and caryophyllene from 11.8 to 41.8%. By adopting gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) techniques, researchers have identified several compounds from essential oil of pepper cultivars and its variability was reported (Gopalakrishnan et al., 1993; Menon et al., 2000, 2002, 2003; Menon and Padmakumari, 2005). Varietal variation of oleoresin and piperine of black pepper was reported by Kurian et al. (2002), Radhakrishnan et al. (2004) and Sasikumar et al. (2004). Purseglove et al. (1981) demonstrated the variation in volatile oil, starch and piperine of two Indian black pepper cultivars in relation to maturity. Variability in primary and secondary metabolites of 26 black pepper cultivars from Peruvannamuzhi and Panniyur (Kerala) was reported by Zachariah et al. (2010). They evaluated total carbohydrate, starch, total free amino acid, total protein, total phenol, essential oil and its constituents, oleoresin and piperine from leaf and berries. They also reported that leaf piperine and leaf phenols showed strong positive correlation with berry piperine. Utpala et al. (2008a) reported maximum variability in β-caryophyllene and nerolidol of leaf oil of *P. nigrum* from Western Ghats of Kerala and Karnataka. Utpala et al. (2008b) studied spatial influence in biochemical constituents of *P. nigrum* L. leaves and found diversity in total phenol, chlorophyll and carotene content in a narrow geographical range.

Black pepper showed variability among the known cultivars with regard to essential oil profile and pungent principles. Panniyur-1 is a leading variety of black pepper at different pepper growing areas of India. Variability of this black pepper variety at different locations assumes great commercial significance. However, a systematic study for its intrinsic quality in relation to location has not been observed. Likewise, information is scanty regarding correlation between chemical constituents of black pepper berries. Hence, the objectives of the present study are to examine variability in constituents in Panniyur-1 black pepper berries in relation to different location and to find out correlation between constituents of Panniyur-1 black pepper berries from different locations. This is the first report on altitudinal variation among secondary metabolites of black pepper and also regarding correlation among constituents like bulk density and starch. Thus the study has great commercial significance.

MATERIALS AND METHODS

Collection of samples

High yielding black pepper variety, Panniyur-1 was freshly harvested during the harvesting season of 2011 and 2012 from eleven locations (Peruvannamuzhi, Ambalavayal, Panniyur, Pampadumpara, Kasaragod, Chelavoor, Appangala, Mudigere, Pechiparai, Thadiankudisai and Dapoli). Immediately after collection, all the samples were sundried to uniform moisture level of 10% and subjected to variability study. Details of eleven locations were illustrated in Table 1.

Physicochemical constituents

Bulk density, total ash and acid insoluble ash were the physico-chemical constituents examined from dried berries of Panniyur-1 from eleven locations by using standard procedures (Gupta and Das, 1997; ASTA, 1968).

Biochemical constituents

Major metabolites such as total carbohydrate, starch, reducing sugars, total free amino acid, total protein, total phenol, Ortho Dihydroxy (OD) phenols, crude fibre and total fat were extracted from dried berries of Panniyur-1 using suitable organic solvents and estimated by adopting standard procedures (ASTA, 1968; Dubois et al., 1956; Eynek et al., 2009; Hodge and Hofreiter, 1962; Johnson and Schaal, 1957; Lowry et al., 1951; Sadasivam and Manickam, 2008; Somogyi, 1952; Yapinlee and Thakahashi, 1966).

Essential oil was estimated by AOAC (1975) method whereas oleoresin and piperine by ASTA (1968) method. Essential oil was extracted from powdered samples by hydrodistillation using Clevenger trap. The oil was quantified and recorded as percentage oil (volume/dry weight in 100 g). Cold percolation technique was used for the extraction of oleoresin. The viscous mass obtained was expressed as oleoresin percentage (weight/dry weight in 100 g). Piperine from the powdered sample was extracted by refluxing in alcohol and estimated by Shimadzu High Performance Liquid Chromatography (HPLC) equipped with SPD-10A UV-visible detector. The column used was Reverse Phase C-18 with a size of 4.6 × 250 mm. Acetonitrile and 1% acetic acid (48:52) were the mobile phase with a flow rate of 1.5 ml/min and measurement was taken at 342 nm. Percentage piperine was computed by using authentic standard (Wood et al., 1988).

GC-MS analysis

Essential oil, extracted by hydro distillation, was subjected to GC-MS analysis for quantification of oil constituents. The oil profile was determined in Shimadzu 2010 GC coupled with MS QP-2010. The separation was done using RtX-5 column and the carrier gas used was helium at 1 ml/min. Column programme: 60°C for 5 min followed by 60 to110°C at 5°C min^{-1}, 110 to 200°C at 3°C min^{-1} and 200 to 220°C at 5°C/min and held for 5 min. The oil constituents were identified by comparison of mass spectra with those in NIST

Table 1. Locations and features.

Location	State	Latitude	Longitude	Altitude (m MSL)
Kasaragod	Kerala	12° 12' 5.97"N	75° 9' 48.51"E	10.7
Chelavoor (Kozhikode)	Kerala	11° 17' 35.16"N	75° 49' 10.2"E	45
Peruvannamuzhi(Kozhikode)	Kerala	11°36' 45" N	75°49'27" E	60
Panniyur (Kannur)	Kerala	12° 4' 35.18"N	75° 24' 19.21"E	95
Ambalavayal (Wayanad)	Kerala	9° 37' 32.46"N	76° 43' 50.52"E	974
Pampadumpara (Idukki)	Kerala	9° 47' 48.12"N	77° 9' 30.89"E	1100
Appangala	Karnataka	12°26'0"N	75°45'0"E	800-1000
Mudigere	Karnataka	13° 7' 54.02" N	75° 38' 27.29" E	1175
Pechiparai	Tamil Nadu	8°14' 23.10"N	77°20'4.02"E	58.3
Thadiankudisai	Tamil Nadu	10°00" N	77°0'0" E	1098
Dapoli	Maharashtra	17° 46' 0.12"N	73° 10' 59.88"E	170-240

Table 2. Physicochemical constituents of Panniyur-1 from different location.

Location	Bulk density (g/L)	Total ash (%) w/w	Acid insoluble ash (%) w/w
Kasaragod	460.60^{h}	5.09^{a}	0.13^{g}
Chelavoor	505.80^{f}	3.89^{ef}	0.27^{c}
Peruvannamuzhi	466.70^{g}	4.17^{d}	0.16^{f}
Panniyur	547.00^{d}	3.87^{f}	0.12^{g}
Ambalavayal	512.00^{e}	3.99^{ef}	0.19^{e}
Pampadumpara	608.70^{a}	4.41^{c}	0.12^{g}
Appangala	556.50^{c}	3.88^{f}	0.07^{i}
Mudigere	573.10^{b}	4.03^{e}	0.09^{h}
Pechiparai	548.90^{d}	3.43^{g}	0.31^{a}
Thadiankudisai	570.70^{b}	4.93^{b}	0.28^{b}
Dapoli	510.60^{ef}	3.45^{g}	0.21^{d}
CD (5%)	5.05	0.14	0.01
CV (%)	6.60	2.26	5.78

and WILEY library and mass spectra in Adams (2007) and quantified by area normalization.

Statistical analysis

Mean value of four replications was used in the study. Data for Panniyur-1 black pepper from eleven locations were combined and analyzed by 'PROC ANOVA' procedure and the correlation between constituents was analyzed by 'PROC CORR' procedure of SAS 9.3 package.

RESULTS

Physicochemical constituents of Panniyur-1 from different location

Variability in physicochemical constituents of Panniyur-1 from different locations is as shown in Table 2. There was clear cut variability among bulk density of samples. Bulk density ranged from 460.6 to 608.7 g/L; it was the highest at Pampadumpara and the lowest at Kasaragod. Ash content and acid insoluble ash had a range of 3.43 to 5.09% and 0.07 to 0.31%, respectively. The highest ash content was observed at Kasaragod and the lowest at Pechiparai and Dapoli. Sample from Pechiparai showed the highest acid insoluble ash whereas that from Appangala had low acid insoluble ash content.

Variability in primary metabolites of Panniyur-1 in relation to location

The major primary metabolites evaluated in the present study were expressed as shown in Table 3. Panniyur-1 showed a significant location wise variability for all primary metabolites. Total carbohydrate ranged from 41.54 to 57.34% whereas reducing sugar ranged from 0.71 to 4.19%. Starch content ranged from 21.57 to 39.46%. Among locations, Panniyur, Kerala had the highest total carbohydrate and reducing sugar whereas

Table 3. Variability in primary metabolites of Panniyur- 1 in relation to location.

Location	Total carbohydrate (%) w/w	Reducing sugars (%) w/w	Starch (%) w/w	Total protein (%) w/w	Total free amino acid (%) w/w	Total fat (%) w/w	Crude fibre (%) w/w
Kasaragod	43.44^{e}	0.89^{gh}	21.57^{f}	9.00^{a}	0.47^{b}	10.34^{a}	18.60^{a}
Chelavoor	48.68^{d}	1.32^{e}	33.08^{d}	6.85^{d}	0.29^{d}	8.12^{e}	15.18^{c}
Peruvannamuzhi	41.54^{e}	1.19^{f}	28.30^{e}	8.23^{b}	0.59^{a}	10.02^{b}	16.86^{b}
Panniyur	57.34^{a}	4.19^{a}	35.87^{bc}	6.28^{e}	0.13^{g}	8.06^{f}	14.62^{d}
Ambalavayal	49.84^{cd}	2.08^{c}	36.15^{bc}	5.43^{f}	0.22^{f}	7.77^{g}	12.54^{f}
Pampadumpara	49.41^{cd}	3.40^{b}	38.91^{a}	4.04^{h}	0.29^{d}	6.16^{k}	10.79^{g}
Appangala	48.58^{d}	1.71^{d}	35.02^{bcd}	4.62^{g}	0.34^{c}	7.68^{h}	14.15^{e}
Mudigere	54.46^{ab}	1.14^{f}	36.28^{b}	7.39^{c}	0.15^{g}	7.30^{i}	14.17^{e}
Pechiparai	53.7^{b}	0.94^{g}	34.12^{cd}	3.27^{i}	0.20^{f}	8.88^{c}	14.61^{d}
Thadiankudisai	52.13^{bc}	0.82^{h}	39.46^{a}	6.85^{d}	0.29^{de}	6.28^{j}	12.45^{f}
Dapoli	52.36^{bc}	0.71^{i}	33.78^{d}	4.62^{g}	0.26^{e}	8.37^{d}	15.19^{c}
CD (5%)	3.00	0.08	1.19	0.18	0.03	0.05	0.39
CV (%)	4.16	3.29	3.93	2.08	5.72	3.20	1.85

Table 4. Variability in secondary metabolites of Panniyur-1 in relation to location.

Location	Essential oil (%) v/w	Oleoresin (%) w/w	Total phenol (%) w/w	Piperine (%) w/w
Kasaragod	2.8^{b}	12.73^{a}	0.63^{a}	4.49^{a}
Chelavoor	2.5^{d}	8.17^{cd}	0.53^{c}	2.76^{c}
Peruvannamuzhi	3.2^{a}	9.74^{b}	0.55^{b}	3.91^{b}
Panniyur	2.0^{f}	7.42^{e}	0.51^{d}	2.55^{d}
Ambalavayal	2.4^{e}	7.14^{ef}	0.40^{f}	2.22^{g}
Pampadumpara	1.6^{h}	6.48^{g}	0.30^{h}	2.13^{h}
Appangala	1.8^{g}	6.91^{f}	0.36^{g}	2.46^{e}
Mudigere	2.0^{f}	7.17^{ef}	0.37^{g}	2.40^{f}
Pechiparai	2.0^{f}	7.98^{d}	0.46^{e}	2.56^{d}
Thadiankudisai	2.4^{e}	5.82^{h}	0.37^{g}	2.40^{f}
Dapoli	2.7^{c}	8.43^{c}	0.46^{e}	2.49^{e}
CD (5%)	0.06	0.40	0.02	0.05
CV (%)	2.13	3.50	1.55	0.92

the highest starch content was observed at Thadiankudisai and Pampadumpara. The lowest total carbohydrate was observed at Kasaragod and Peruvannamuzhi and for reducing sugar and starch it was Dapoli and Kasaragod, respectively. Total protein and total free amino acid of Panniyur-1 from eleven locations ranged from 3.27 to 9.0% and 0.15 to 0.59%, respectively. The highest protein content was found at Kasaragod whereas the lowest was at Pechiparai. Total free amino acid content was high at Peruvannamuzhi whereas Mudigere and Panniyur showed lowest total free amino acids. The total fat content had a range of 6.16-10.34%. Highest fat content was recorded at Kasaragod whereas the lowest at Pampadumpara. Crude fibre was in the range of 10.79 to 18.6% and it was the highest at Kasaragod and the lowest at Pampadumpara.

Variability in secondary metabolites of Panniyur-1 in relation to location

Table 4 illustrates the level of the major secondary metabolites in Panniyur-1. The essential oil content varied between 1.6 and 3.2%. The highest oil content was recorded at Peruvannamuzhi and the lowest at Pampadumpara. Oleoresin in Panniyur-1 berries ranged from 5.82 to 12.73%. Kasaragod showed the highest oleoresin content whereas Thadiankudisai had the lowest oleoresin content. Piperine, the pungent principle of pepper, was in the range of 2.13 to 4.49%. Piperine content was the highest at Kasaragod and the lowest at Pampadumpara. The total phenol content had a range of 0.30 to 0.63%. The highest phenol content was observed at Kasaragod and the lowest at Pampadumpara.

Oil profile by GC-MS

Forty nine compounds were identified from the essential oil of Panniyur-1 from different locations and the oil profile is as shown in Table 5. Major monoterpenes identified in the pepper oil were thujene, pinene, sabinene, myrcene, limonene and terpinenes, whereas the major sesquiterpene was β-caryophyllene. Alpha-phellandrene, carene, cymene, ocimene, germacrene-D, linalool, copaene, cubebene, humulene, bergamotene, guaiene, etc., were the other compounds present in pepper oil. Beta-caryophyllene content ranged from 9.52 to 26.95% and it was the highest at Pechiparai and the lowest at Pampadumpara with an altitudinal range of 58.3 to 1100 m MSL. The significant finding was that clear altitudinal variability was observed for β-caryophophyllene at different altitudes. β-caryophophyllene was low at high altitudes (>500 m MSL) and high at low altitudes. This kind of variability was not observed with other sesquiterpenes. Similarly, monoterpenes like thujene, α-pinene, sabinene, limonene, α-phellandrene and linalool were relatively high at higher altitudes as compared to plains. Statistical analysis was performed only for those constituents that showed significant altitudinal variation.

Correlation between constituents

Apart from biochemical variability in Panniyur-1 at different location, significant correlation was observed between many constituents. Table 6 illustrates the coefficient of correlation (r) for each interaction among constituents. The correlation for different metabolites of black pepper berries observed in the present study has not been reported by any worker so far. Bulk density was positively correlated with starch (r=+0.83) and had negative correlation with total phenol, piperine, oleoresin, crude fibre, essential oil and total fat. Total phenol, essential oil, piperine and oleoresin showed positive correlation with each other and also with crude fibre and total fat, but negatively correlated with bulk density and starch. There was a positive correlation between crude fibre and total fat.

DISCUSSION

This study concentrated on the impact of location on the physical and chemical constituents of black pepper. Considering the commercial importance of black pepper variety Panniyur-1 which is grown at all major pepper growing areas, bulk density assumes great significance. The study clearly demonstrated that bulk density of berries from some of the locations like Pampadumpara, Mudegere and Thadiankudisai were comparatively high. Jayashree et al. (2009) reported that bulk density of 'Panniyur-1' from IISR Experimental Farm, Peruvannamuzhi, kerala had a range of 454.7 to 513.3 g/L in relation to berry size. The high ash content is the reflection of mineral contents preserved in the sample (Antia et al., 2006). Zachariah and Parthasarathy (2008) reported the limits for total ash and acid insoluble ash as 5 and 0.5%, respectively. The samples from all the locations in the present study adhere to this limit. The total ash and acid insoluble ash of black pepper berries collected from local market was reported by Kolhe et al. (2011) as 1 and 0.55%, respectively.

Primary metabolites are responsible for growth and development of the plant (Shamina and Sharma, 2001). Total carbohydrate, reducing sugar, starch and total protein influence the productivity of black pepper and also provide precursors for piperine biosynthesis. Factors like temperature, rate of photosynthesis, etc., influencing the carbohydrate and protein level of black pepper berries (Prejeena, 2003; Sumesh, 2004; Shujari, 2005). Thus, in the present study, variability in primary metabolites may be due to variation in the aforementioned factors with respect to locations. However, the values from all locations fall within the reported limits. Since total free amino acids tend to change during disease, their measurements will give an idea about physiological and health status of the plants (Sadasivam and Manickam, 2008). Zachariah et al. (2010) reported that total carbohydrate of dried berries of 26 black pepper cultivars from Panniyur and Peruvannamuzhi, Kerala was in the range of 38.6 to 51.2% whereas starch, total free amino acid and total protein ranged from 32.1 to 43.2%, 0.3 to 0.8%, and 2.1 to 6.0%, respectively.

Secondary metabolites in black pepper are mainly responsible for defense mechanism, pungency and aroma (Shamina and Sharma, 2001). The variation in major secondary metabolites of Panniyur-1 in relation to location is as shown in Table 4. All the secondary metabolites showed significant location wise variation. It was clear from Table 4 that locations such as Kasaragod, Chelavoor, Pechiparai, Panniyur and Peruvannamuzhi showed relatively more total phenol as compared to the remaining high altitude locations. Apart from variation among location, total phenol showed a significant altitudinal variation also. This is the first report regarding altitudinal variation of total phenols in black pepper berries. Phenol content has a major role for disease resistance in plants. Precursors of secondary metabolic pathways are products of the primary metabolism. Therefore a severe or long lasting stress factor could induce an excessive shift between primary and secondary metabolism and consequently a diversion of available resources from growth to defense (Iriti and Faoro, 2009). These factors also can be attributed for accounting the variability in total phenol at different locations. Blackening of pepper occurs as a result of oxidation of OD phenols by *o*-diphenol oxidase. Therefore, conversion of green pepper to black pepper results in the complete loss of OD phenols and 75% loss in total phenols (Bandyopadhyay et al., 1990). In the

Table 5. Oil profile of dried berries of Panniyur-1 black pepper.

Compound	Composition (%)										
	K	C	Z	R	L	P	A	M	E	G	D
α-Thujene	**0.60^{j}**	**0.83^{i}**	**1.24^{f}**	**0.86^{h}**	**2.61^{c}**	**2.30^{e}**	**2.65^{b}**	**2.53^{d}**	**0.47^{k}**	**2.94^{a}**	**1.03^{g}**
α-Pinene	**3.88^{k}**	**4.18^{h}**	**4.62^{f}**	**3.96^{j}**	**5.77^{b}**	**4.52^{g}**	**5.19^{d}**	**5.73^{c}**	**4.00^{i}**	**6.48^{a}**	**4.86^{e}**
Camphene	0.14	0.12	0.12	0.09	0.14	0.15	0.15	0.11	0.09	0.15	0.08
Sabinene	**0.00^{k}**	**8.09^{i}**	**10.01^{f}**	**8.35^{h}**	**17.76^{c}**	**14.60^{e}**	**15.9^{d}**	**18.07^{b}**	**4.31^{j}**	**19.23^{a}**	**8.64^{g}**
β-Pinene	13.26	6.82	6.96	8.93	7.76	4.67	5.16	9.90	10.21	8.98	10.65
β –Myrcene	1.76	2.27	2.22	2.34	2.62	2.28	2.28	2.06	1.95	2.74	2.20
α-Phellandrene	**0.00^{h}**	**0.20^{f}**	**0.26^{e}**	**0.17^{g}**	**0.52^{c}**	**1.04^{a}**	**0.52^{c}**	**0.50^{d}**	**0.16^{g}**	**0.58^{b}**	**0.16^{g}**
δ-3-Carene	-	0.12	0.04	-	-	4.40	0.66	1.08	0.27	-	-
α-Terpinene	0.07	-	0.16	0.06	0.23	0.28	0.45	0.11	0.06	0.38	0.07
p-Cymene	-	-	-	-	-	-	-	0.81	-	-	
D-Limonene	**15.13^{k}**	**15.99^{i}**	**15.75^{j}**	**16.60^{g}**	**19.53^{b}**	**17.70^{e}**	**18.4^{d}**	**19.33^{c}**	**16.6^{h}**	**20.78^{a}**	**17.4^{f}**
Z-Ocimene	0.03	0.14	0.01	0.14	0.02	0.21	0.01	0.09	0.06	0.02	0.09
E-Ocimene	-	-	0.16	-	0.23	-	0.19	-	-	0.22	-
γ-Terpinene	0.03	0.22	0.28	0.12	0.42	0.71	0.84	o.28	0.11	0.68	0.14
α-Terpinolene	0.04	0.25	0.25	0.19	0.29	0.49	0.41	0.19	0.16	0.34	0.15
β –Linalool	**0.39^{g}**	**0.41^{f}**	**0.47^{e}**	**0.33^{h}**	**0.74^{d}**	**1.70^{a}**	**0.86^{c}**	**0.96^{b}**	**0.23^{i}**	**0.87^{c}**	**0.21^{j}**
trans-p-Menth-2-en-1-ol	-	0.03	-	-	-	0.35	0.23	0.13	-	0.17	-
L-4-Terpineol	0.71	0.57	0.81	0.57	1.19	3.62	2.91	2.09	0.32	1.95	0.47
Cryptone	0.07	-	-	-	-	0.16	0.07	0.11	-	-	-
α- Terpineol	0.18	0.22	0.17	0.01	0.19	0.40	0.24	0.22	0.13	0.18	0.10
trans-Piperitone	-	-	-	-	-	0.02	0.04	-	-	-	-
Nerol	0.04	0.03	-	-	-	0.02	-	-	-	-	-
δ-Elemene	1.36	2.27	2.41	1.03	0.88	0.40	0.70	0.42	2.10	0.77	1.20
α-Cubebene	0.49	0.12	0.45	0.39	0.41	0.37	0.47	-	0.38	0.32	0.26
α-Copaene	4.75	0.20	5.19	5.31	4.79	3.06	4.65	3.92	5.51	3.91	5.06
β- Cubebene	-	-	-	-	0.62	-	-	0.16	-	-	-
β- Elemene	1.06	0.69	0.91	0.59	0.38	0.51	0.42	0.08	0.63	0.21	0.50
α-Gurjunene	0.07	1.10	0.18	-	0.14	0.09	0.16	-	-	0.07	-
β-Caryophyllene	**21.41^{e}**	**21.9^{d}**	**20.28^{f}**	**22.99^{c}**	**13.49^{g}**	**9.52^{k}**	**12.8^{h}**	**12.48^{i}**	**26.95^{a}**	**10.71^{j}**	**26.3^{b}**
β-Farnesene	0.47	0.36	-	-	-	-	-	-	-	-	-
α-Bergamotene	-	-	-	-	-	0.07	-	-	-	-	-
α-Guaiene	-	-	-	-	-	0.04	-	-	-	-	-
α-Humulene	2.43	0.83	2.02	2.15	1.16	1.53	1.24	1.26	3.03	1.11	2.44
γ-Muurolene	0.17	0.07	0.12	0.11	0.06	0.04	0.06	-	0.12	0.02	-
Germacrene-D	-	0.10	0.44	0.52	0.32	0.57	0.37	-	0.42	0.23	0.35
β-Selinene	0.68	0.49	0.90	0.57	0.64	0.87	0.63	0.52	0.63	0.39	0.57
α-Zingeberene	-	0.97	0.39	0.14	0.20	0.14	0.24	0.07	0.20	0.17	0.12
α-Selinene	-	0.30	1.52	0.45	0.52	0.94	0.85	-	-	-	0.43
β-Bisabolene	6.49	1.32	7.05	6.91	7.03	5.68	7.96	7.07	7.08	7.58	5.98
γ-Cadinene	-	0.03	-	-	-	1.01	1.6	-	-	-	-
δ-Cadinene	2.43	1.82	2.47	2.36	2.12	1.74	2.63	1.49	2.59	1.83	2.14
t-Nerolidol	0.16	0.18	0.06	0.07	0.08	0.97	0.07	-	0.09	-	-
Caryophyllene oxide	4.91	0.06	0.51	0.83	0.54	1.56	1.00	1.32	0.86	0.16	0.73
δ-Cadinol	0.35	0.16	0.16	0.12	0.12	0.19	0.24	-	0.14	0.23	-
Spathulenol	2.31	-	0.14	0.15	0.10	0.71	0.48	0.26	0.14	-	0.10
α-Cadinol	4.89	3.84	0.74	0.73	0.22	0.36	3.06	0.18	0.36	0.19	0.23
Amorphan-3en-9-ol	-	0.64	3.89	4.29	2.16	2.40	-	2.15	3.59	1.97	2.29
Pentadecanal	0.14	-	0.12	0.22	0.20	0.09	0.12	0.12	0.17	0.16	0.12
Nonadecanol	0.08	0.29	-	-	0.06	0.08	0.06	-	-	-	-

K=Kasaragod, C=Chelavoor, Z=Peruvannamuzhi, R=Panniyur, L=Ambalavayal, P=Pampadumpara, A=Appangala, M= Mudigere, E= Pechiparai, G= Thadiankudisai, D=Dapoli. Compounds showed significant altitudinal variation were expressed in bold.

Table 6. Correlation matrix (p<0.05).

Matrix	P	TP	O	BD	S	CF	EO	TF
P	1.00							
TP	+0.83	1.00						
O	+0.92	+0.86	1.00					
BD	-0.80	-0.87	-0.82	1.00				
S	-0.93	-0.84	-0.95	+0.83	1.00			
CF	+0.88	+0.90	+0.89	-0.83	-0.90	1.00		
EO	+0.71	+0.71	+0.64	-0.88	-0.64	+0.68	1.00	
TF	+0.85	+0.88	+0.89	-0.83	-0.89	+0.92	+0.68	1.00

P=Piperine; TP=Total Phenol; O=Oleoresin; BD=Bulk density; S=Starch; CF=Crude fibre; EO=Essential oil; TF=Total fat.

present work, OD phenols were not detected in any of the samples. This observation was supported by Bandyopadhyay et al. (1990). It was observed that Panniyur-1 from Kasaragod and Peruvannamuzhi had high secondary metabolites (essential oil, oleoresin, total phenol, and piperine) while sample from Pampadumpara and Thadiankudisai showed relatively low values for same constituents.

Essential oil extracted from samples was subjected to GC-MS analysis for identification of oil constituents. Forty nine compounds were identified from essential oil samples and expressed in Table 5. Good variability was observed among essential oil constituents in the present study. Significantly, clear altitudinal variability was obtained for β-caryophophyllene; it was low at high altitudes (>500 m MSL) and high at low altitudes. Similarly, monoterpenes like thujene, α-pinene, sabinene, limonene, α-phellandrene and linalool were relatively high at higher altitudes compared to plains. Jagella and Grosch (1999) demonstrated that pinene, limonene, α-phellandrene, linalool, etc., are the potent odorants of pepper oil. In the present study, these constituents are relatively high at higher altitudes and it can be concluded that aroma profile is superior at higher altitudes.

Correlation between constituents

Correlation between constituents of Panniyur-1 from eleven locations was also performed. Significant correlation was observed between many constituents (Table 6).

Correlation between bulk density and metabolites

Bulk density is the major physical property of biomass and it influences directly the cost of the substances (Lam et al., 2008). It also impacts storage requirements, the sizing of the material handling system and how the material behaves during subsequent thermo-chemical and biological processes (McKendry, 2002). The size, shape, moisture content, individual particle density, surface characteristics, etc., are few factors affecting bulk density (Lam et al., 2008). In this study, bulk density was positively correlated with starch (r=+0.83) and had negative correlation with total phenol, piperine, oleoresin, crude fibre, essential oil and total fat. A positive correlation between bulk density and starch content was reported by Fife et al. (2008) in barley grains.

Correlation among metabolites

Starch, the important reserved carbohydrate showed significant negative correlation with metabolites, namely, total phenol, piperine, oleoresin, essential oil, total fat and crude fibre. Total phenol, an important secondary metabolite for disease resistance in plants had positive correlation with piperine, oleoresin, essential oil, crude fibre and total fat. Piperine, the major pungent principle in black pepper had same pattern of correlation as that of total phenol. A positive correlation was shown by oleoresin with total phenol, piperine, crude fibre, essential oil and total fat. Positive correlation was also observed between crude fibre and essential oil; crude fibre and total fat; essential oil and total fat. This is the first report for correlation among different metabolites of black pepper berries. Starch had significant negative correlation with metabolites, namely, total phenol, piperine, oleoresin, essential oil, total fat and crude fibre.

Conclusion

It can be concluded that there was a significant location wise variation for both primary and secondary metabolites of Panniyur-1 black pepper berries. Variability was profound in essential oil, oleoresin, piperine, total phenol, crude fibre, starch, total fat and bulk density. Except total phenol and β-caryophyllene of essential oil, no other constituents showed altitudinal specificity. These two constituents were low at high elevations (>500 MSL) and high at plains. Similarly, monoterpenes like thujene, α-pinene, sabinene, limonene, α-phellandrene and linalool

were relatively high at higher altitudes as compared to plains. Desirable aroma profile as per Jagella and Grosch (1999) holds good in case of higher altitude black pepper oil samples. The study also revealed that there was a significant correlation between many constituents of Panniyur-1 from different locations. This is the first report for correlation among metabolites of black pepper berries. Hence, this study assumes great commercial significance.

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