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Quantitative estimation of polyphenols among *terminalia-arjuna populations* collected from different sites of Chhattisgarh and Madhya Pradesh, India

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Abstract

The objective of the present work was to assess variation in total phenolic content in Terminalia arjuna populations from different locations of Madhya Pradesh and Chhattisgarh. Different regions of Madhya Pradesh and Chhattisgarh were surveyed and bark samples from phenotypically superior population was collected. A spectrophotometric method was used for quantification of phenols using Folin-Ciocaltue reagent. The results were expressed as milligrams of gallic acid equivalent per gram of dry weight (GAE g/dw). Significant (P=0.01) *variation was observed in polyphenols in different populations of T.arjuna. The quantity of polyphenols varied 12.20 to 15.94%. Among six selected populations, phenolic content was found to be maximum in Bilaspur, CG germplasm (15.94mg/100gm) followed by Mandla, MP (15.07mg/100gm). Seoni, MP, germplasm contained the minimum amount of phenolic content (12.20 mg/100gm). A significant correlation was found between the bark thickness and phenolic contents. The populations with high phenolic contents can be utilized for mass multiplication and genetic improvement.*

Keywords: *Terminalia arjuna*, populations, phenotypically superior, spectrophotometric method, polyphenols.

Introduction

Terminalia arjuna [Roxb. Ex DC] Wight and Arnot is a large tree of the flowering plant family Combretaceae, distributed in tropical regions of the world. The tree is common throughout the greater part of the Indian peninsula along rivers, streams, ravines and dry watercourses, found in sub-Himalayan tract, Andhra Pradesh, Assam, Bihar, Gujarat, Jammu and Kashmir, Maharashtra, Karnataka, Tamil Nadu, Utter Pradesh and West Bengal[1,2]. The stem bark of tree contains large amount of polyphenolic compounds and used as anti-oxidant, antibiotic[3], astringent, cooling, aphrodisiac cardio tonic[4], in fractures, ulcers, spermatorrhae, leucorrhea, diabetes, cough, tumor, excessive perspiration, asthma, inflammation, hypocholesteremia[5] and skin disorders[6,7] also shows antimutagenic activity[8]. The dried stem bark has been used widely as a drug in Ayurvedic medicine as a cardio tonic (hridya), for injury or wound (kshata), emaciated condition (kshaya), poison (visha), blood disorders (raktavikāra), obesity (medaroga), urinary disorders (prameha) and ulcer or wound (vrana)[9,10]. The use of arjun bark powder as an astringent and diuretic finds mention in Charaka Samhita. Vagbhata, a disciple of Charaka, was the first to recognize the cardio-protective property of the bark in his treatise Ashtānga Hridayam some 1200 years ago[11]. It decreases LDL levels and an excellent medicine for the heart. It has the capability to even reverse heart failure[12,13] and has been traditionally used to treat heart disease for centuries, getting nickname "Guardian of the heart" and derived name "Arjun"- the hero of the famous epic "Mahabharata"[14-17]. Keeping the above facts in mind, the present work was undertaken to assess variation in total phenolic content in *Terminalia arjuna* populations from different locations of Madhya Pradesh and Chhattisgarh.

Materials and methods

Collection and preparation of crude extract: *Terminalia arjuna*bark samples were collected from different sites i.e., Bilaspur, Ambikapur, Raipur (Chhattisgarh) Balaghat, Mandla, andSeoni (Madhya Pradesh) on the basis of GPS location and phenotypic character.

The collected materials were washed with distilled water to expel the residue particles and microbes. Samples were shade dried at room temperature. The dried plant materials were ground to a coarse. The amount of total phenolic content was determined by Folin-Ciocalteau's method as described by Lister and Wilson[18].

Accurately weighed 100mg of crude sample and grind it with a mortar and pestle in 10times volume of 80% ethanol and transferred to a 50mL centrifuge tube. Centrifuged at 10,000 rpm for 20min and save the supernatant. Re-extracted the residue with five the volumes of 80% ethanol, centrifuged, and pool the supernatant. Evaporated the supernatant to dryness. Dissolved the supernatant in 5ml of distilled water. Pipette out three aliquots, containing 0.2ml, into test tubes to 3ml with water. Added 0.5ml of Folin-Ciocalteau's reagents. After 3min, added 2ml of 20% Na_2CO_3 solution to each tube and incubated at 45°C for 15min. The absorbance of all samples was measured at 765nm using UV-Vis spectrophotometer against blank. The results were expressed as milligrams of gallic acid equivalent per gram of dry weight (GAE g/dw). From the standard curve, the concentration of total phenol in test samples was expressed as mg phenols/100g material.

Calculation

$$\text{Concentration} = \frac{\text{Conc. of Standard} \times \text{ OD of Sample } \times \text{ Total vol make up } \times 100}{\text{OD of standard} \times \text{ weight of sample } \times \text{ Vol taken}}$$

Table-1: Details of collection sites with GPS locations.

Location	Tree no	GPS Location		Elevation (m)	Plant girth (cm)	Bark Thickness (mm)
		N	E			
Bilaspur	1	24°52' 42.1"	081°11' 27.8"	603	220	18
	2	24°49' 49.2"	082°10' 03.1"	602	186	17
	3	22°02' 29.2"	084°48' 00.2"	587	132	17
	4	22°59' 42.1"	082°15' 52.5"	577	138	22
	5	23°46' 51.9"	082°08' 33.5"	573	148	17
	6	24°03' 41.8"	081°20' 52.4"	324	183	15
	7	24°27' 16.6"	080°11' 55.8"	570	180	13
	8	23°16' 32.0"	081°13' 49.3"	442	152	22
	9	23°32' 13.2"	081°20' 30.3"	442	128	16
	10	24°50' 47.7"	083°24' 50.3"	305	125	13
	11	22°18' 04.9"	083°14' 13.9"	467	138	16
	12	20°58' 12.2"	082°15' 51.2"	332	126	18
	13	21°51' 03.8"	078°12' 20.2"	366	125	15
	14	29°20' 13.0"	078°51' 32.2"	338	133	16
	15	24°23' 46.3"	081°18' 40.0"	337	125	10
	16	23°20' 52.9"	079°50' 21.2"	368	162	25
	17	23°02' 49.4"	079°49' 56.9"	442	147	12
	18	24°50' 47.7"	083°24' 50.3"	305	137	24
	19	23°30' 17.9"	080°47' 15.0"	487	148	18
	20	23°39' 17.7"	080°38' 34.3"	389	202	16
Raipur	1	25°14' 17.6"	080°43' 47.2"	319	134	16
	2	21°02' 45.7"	082°07' 04.7"	313	135	6
	3	21°38' 41.4"	080°18' 15.0"	287	126	18
	4	25°14' 16.2"	081°06' 14.6"	169	126	14
	5	23°35' 27.3"	082°29' 48.2"	299	127	16
	6	23°44' 25.8"	082°39' 26.9"	299	130	20
	7	21°38' 48.5"	082°08' 45.5"	297	183	18

	8	21°08' 02.6"	081°44' 37.3"	320	153	15
	9	21°08' 57.8"	081°44' 37.3"	317	155	16
	10	21°07' 58.1"	081°44' 50.4"	317	152	10
	11	21°07' 58.7"	081°44' 50.6"	317	144	10
	12	21°07' 58.7"	081°44' 50.7"	316	140	13
	13	21°07' 58.7"	081°44' 50.7"	317	124	13
	14	21°07' 59.0"	081°44' 50.8"	315	136	14
	15	21°07' 59.2"	081°44' 50.4"	319	124	13
	16	21°07' 59.3"	081°44' 50.3"	319	125	15
	17	21°07' 58.6"	081°44' 50.1"	319	127	8
	18	21°08' 03.0"	081°44' 50.1"	317	125	15
	19	21°08' 03.8"	081°44' 50.4"	316	134	19
	20	21°08' 03.9"	081°44' 50.8"	318	153	17
Mandla	1	22°38' 30.1"	080°21' 27.5"	461	163	8
	2	22°38' 30.0"	080°21' 26.4"	449	208	13
	3	22°38' 30.2"	080°21' 26.2"	450	152	10
	4	22°38' 31.5"	080°21' 25.4"	451	156	9
	5	22°38' 31.9"	080°21' 24.9"	452	202	8
	6	22°38' 32.6"	080°21' 23.6"	454	246	11
	7	22°38' 33.2"	080°21' 22.1"	453	230	10
	8	22°38' 33.3"	080°21' 28.0"	451	183	14
	9	22°38' 34.5"	080°21' 20.5"	453	170	6
	10	22°38' 34.9"	080°21' 20.1"	454	156	10
	11	22°38' 35.0"	080°21' 19.9"	454	166	9
	12	22°38' 35.8"	080°21' 19.4"	452	202	8
	13	22°38' 36.4"	080°21' 18.6"	454	233	6
	14	22°38' 35.9"	080°21' 18.7"	454	204	10
	15	22°39' 02.4"	080°20' 41.0"	456	196	12
	16	22°39' 02.6"	080°20' 40.7"	454	182	11
	17	22°39' 02.7"	080°20' 40.7"	455	130	8
	18	22°39' 02.7"	080°20' 40.4"	453	131	9
	19	22°39' 03.0"	080°20' 40.5"	455	138	6
	20	22°39' 03.4"	080°20' 38.3"	455	212	8

Balaghat	1	22°11' 28.7"	080°07' 37.9"	362	161	12
	2	22°11' 28.8"	080°07' 37.2"	361	127	14
	3	22°11' 29.4"	080°07' 37.3"	365	182	12
	4	22°11' 30.4"	080°07' 37.5"	365	181	10
	5	22°11' 31.0"	080°07' 37.2"	368	203	16
	6	22°11' 31.7"	080°07' 36.6"	368	206	9
	7	22°11' 32.1"	080°07' 36.4"	367	176	11
	8	22°11' 32.8"	080°07' 35.9"	369	160	11
	9	22°11' 33.5"	080°07' 35.6"	371	182	13
	10	22°11' 34.4"	080°07' 35.1"	367	135	10
	11	22°11' 34.6"	080°07' 35.1"	367	124	14
	12	22°11' 35.9"	080°07' 34.7"	366	166	13
	13	22°11' 36.4"	080°07' 34.7"	364	155	17
	14	22°11' 36.4"	080°07' 34.2"	365	207	15
	15	22°11' 36.8"	080°07' 34.4"	365	172	18
	16	22°11' 36.9"	080°07' 34.1"	366	214	15
	17	22°11' 38.0"	080°07' 32.3"	369	206	14
	18	22°11' 37.7"	080°07' 32.7"	371	214	9
	19	22°11' 38.9"	080°07' 31.3"	374	160	18
	20	22°11' 38.9"	080°07' 31.1"	368	185	15
Seoni	1	21°49' 16.1"	079°40' 30.8"	464	152	10
	2	21°49' 16.8"	079°40' 33.8"	476	235	13
	3	21°49' 14.8"	079°40' 30.1"	461	269	23
	4	21°49' 14.6"	079°40' 29.6"	463	122	7
	5	21°53' 12.0"	079°48' 32.7"	557	125	10
	6	21°53' 11.8"	079°48' 32.5"	553	116	10
	7	21°53' 11.2"	079°48' 32.1"	552	131	11
	8	21°53' 09.3"	079°48' 32.1"	552	131	16
	9	21°53' 09.3"	079°48' 32.2"	552	139	13
	10	21°53' 09.4"	079°48' 32.5"	552	172	14
	11	21°53' 09.9"	079°48' 31.5"	553	178	18
	12	21°53' 10.8"	079°48' 30.5"	543	170	16
	13	21°53' 11.7"	079°48' 30.3"	560	182	18

	14	21°53' 11.7"	079°48' 30.4"	559	134	22
	15	21°53' 12.0"	079°48' 30.0"	557	162	20
	16	21°53' 12.6"	079°48' 29.8"	556	212	13
	17	21°53' 12.7"	079°48' 29.8"	558	190	20
	18	21°53' 13.4"	079°48' 29.1"	554	145	16
	19	21°53' 13.5"	079°48' 28.6"	558	178	14
	20	21°53' 13.4"	079°48' 28.3"	556	136	16
Ambikapur	1	23°17' 57.5"	083°20' 07.2"	539	292	13.93
	2	23°17' 58.9"	083°20' 06.8"	549	240	12.47
	3	23°17' 59.6"	083°20' 06.5"	549	160	15.23
	4	23°17' 58.7"	083°20' 10.3"	557	175	16.3
	5	23°18'00.9"	083°20' 08.9"	559	192	18.6
	6	23°18'01.5"	083°20' 08.5"	559	230	23.24
	7	23°18'02.1"	083°20' 08.2"	559	178	20.28
	8	23°18'02.5"	083°20' 07.9"	555	112	12.49
	9	23°18'02.6"	083°20' 07.9"	554	120	14.88
	10	23°18'02.7"	083°20' 07.6"	556	211	18.43
	11	23°18'03.1"	083°20' 07.7"	553	254	20.16
	12	23°18'03.3"	083°20' 07.5"	554	292	36.3
	13	23°18'03.7"	083°20' 07.9"	554	130	22.39
	14	23°18'59.7"	083°20' 08.2"	554	185	7.69
	15	23°18'59.6"	083°20' 09.2"	552	165	11.09
	16	23°18'56.7"	083°20' 10.2"	547	216	8.82
	17	23°18'57.3"	083°20' 10.0"	547	280	14.79
	18	23°18'55.2"	083°20' 10.5"	548	125	10.48
	19	23°14'27.8"	083°18' 44.9"	576	208	22.2
	20	23°14'26.9"	083°18' 45.51	577	135	11.61

Results and discussion

The results of polyphenols in different *T.arjuna* populations,bark thickness and diameter are depicted in Table-1 and 2. Significant (P=0.01) variation was observed in polyphenols in different populations of *T.arjuna* (Figure-1). The quantity of polyphenols varied 12.20 to 15.94%. Among six selected populations, phenolic content was found to be maximum in Bilaspur (C.G.) germplasm (15.94mg/100gm) followed by Mandla (M.P.) (15.07mg/100gm). Seoni (M.P.) germplasm contained the minimum amount of phenolic content (12.20 mg/100gm). A significant correlation was found between the bark thickness and phenolic contents (Table-3).

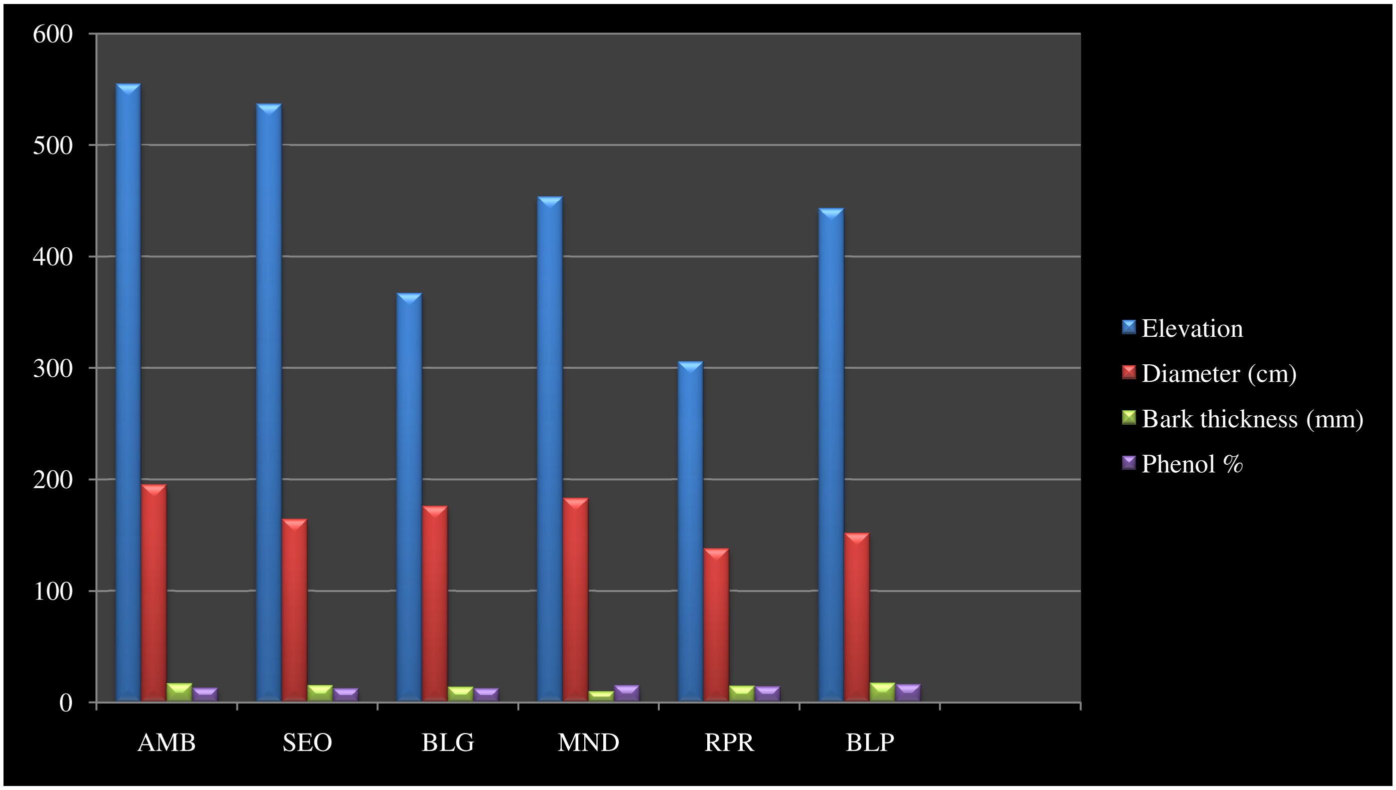

Figure-1: Diameter, bark thickness and phenol contents of mapping population of Arjun (Abbr. AMB=Ambikapur, SEO=Seoni, BLG=Balaghat, MND=Mandla, RPR=Raipur and BLP= Bilaspur).

Table-2: Total phenolic content, diameter and bark thickness of different samples

Locations	Elevation	Diameter (cm)	Bark thickness (mm)	Phenol %
AMB	554.9	195.00	16.56	12.62
SEO	536.8	163.95	15.00	12.20
BLG	366.9	175.80	13.30	12.23
MND	453.5	183.00	9.30	15.07
RPR	305.5	137.65	14.30	13.95
BLP	442.8	151.75	17.00	15.94

AMB= Ambikapur, SEO= Seoni, BLG= Balaghat, MND= Mandla, RPR= Raipur and BLP=Bilaspur.

Table-3: Simple correlation coefficients among four parameters in Arjun population.

Parameters	Elevation	Diameter	Bark Thickness	Phenol content
Elevation	-	0.283	-0.091	0.569
Diameter	0.283	-	-0.579	0.694
Bark Thickness	-0.091	- 0.579	-	-0.475
Phenol content	-0.569	-0.694	-0.475	-

The phenol concentration was determined by the standard graph of catechol and it was found to be maximum i.e. 15.94 in the samples collected from Bilaspur and minimum in the samples collected from Seoni i.e. 12.20, of powdered plant material. A significant correlation was found to be between bark thickness and phenolic contents. There was no correlation was found between the diameter and bark thickness.

Discussion: Polyphenols are complex secondary metabolites, physiologically active against herbivores or pathogens, having various medicinal properties. The investigation of plant phenolics has deciphered in its various practical applications. Several previous studies have revealed the impact of plant phenolics on the mechanism against diseases and pathogens. Apart from this, it has been also determined that these substances have significant roles in various physiological activities in the plants including growth vigor, differentiation of flowers and roots, determination of gene activity and characterization of some developmental stages[19-21]. In this study, we found phenolic contents increase with the increase in thickness of barks, however, some exceptions are also observed. In the case of the population collected from the Mandla has detected with minimum thickness while its phenolic content is found higher as compared to the other populations.

Conclusion

Finally, we concluded that the population collected from Bilaspur has the maximum bark thickness with the highest phenolic content and Seoni has the lowest phenolic content. Bilaspur is the district which is covered under the northern hill regions of Chhattisgarh with the annual mean rainfall of 1292 mm. It might be possible that the trees from which samples were collected suffer from more outbreaks of biotic and abiotic stress; it might be a reason for more phenolic contents production by the plants which we depict as their defense mechanism against pathogens. High the phenolic content is known to be associated with higher the antioxidant activity. As the phenolic content increase, antioxidant activity increases as well. *Terminalia arjuna* viewed as promising plant species for natural plant sources of antioxidants with high potential value for drug preparation. The genetic diversity analysis of genus *Terminalia* has taken speed from last one decade as the species belonging to this genus have very high economic importance. These species need an undivided attention for their conservation and management due to their very high rate of exploitation for medicinal purpose.

References

1. Warrier P.K., Nambiar V.P.K. and Ramankutty C. (1996). Terminalia arjuna. *Indian Medicinal Plants—A Compendium of*, 500, 253-257.
2. Nandkarni A.K. (1976). Indian Materia Medica. IstEdn., Popular Prakshan, Mumbai, India.
3. Perumal S.R., Ignacimuthu S. and Sen A. (1998). Screening of 34 Indian medicinal plants for antibacterial properties. *J.Ethnopharmacol.*, 62(2), 173-181.
4. Karthikeyan K., Bai B.R., Gauthaman K., Sathish K.S. and Devaraj S.N. (2003). Cardio protective effect of the alcoholic extract of *Terminalia arjuna*bark in an in vivo model of myocardial ischemic reperfusion injury. *Life Science*, 73(1), 2727-2739.
5. Miller A.L. (1998). Botanical influences on cardiovascular disease. *Altern Med Rev*, 3(6), 422-431.
6. Dwivedi S. and Agarwal M.P. (1994). Antianginal and cardio protective effect of *T. arjuna*, an indigenous drug, in coronary artery disease. *J. Assoc. Physician India*, 42, 287-289.
7. Raghavan B. and Kumari S.K. (2006). Effect of *T. arjuna*stem bark on antioxidant status in liver and Kidney of alloxan diabetic rats. *Indian J. Physio.Pharmacol.*, 50(2), 133-142.
8. Scassellati-Sforzolini G., Villarini L.M., Moretti L.M., Marcarelli L.M., Pasquini R., Fatigoni C., Kaur L.S., Kumar S. and Grover I.S. (1999). Antigenotoxic properties of *Terminalia arjuna*bark extracts. *J.Env. Path.Tox.Oncol.,* 18(2), 119-125.
9. Rose J. and Treadway S. (2000). Herbal support for a healthy cardiovascular system. *ClinNutr Insights*, 6(16), 1-6.
10. Khan S. and Balick M.J. (2001). Therapeutic plants of ayurveda: a review of selected clinical and other studies for 166 species. *J.Altern.Compliment. Med.*, 7(5), 405-515.
11. Udupa K.N. (1986). Scope of use of Terminalia arjuna in ischaemic heart disease. *Ann Natl Acad Indian Med*, 1(1), 54-58.
12. Bharani A., Ganguli A. and Bhargava K.D. (1995). Salutary effect of *Terminalia arjuna* in patients with severe refractory heart failure. *Int J.Cardiol.*, 49(3), 191-199.
13. Dwivedi S. and Jauhari R. (1997). Beneficial effects of Terminalia arjuna in coronary artery disease. *Indian Heart Journal*, 49(5), 507-510.
14. King F.E., King T.J. and Ross J.M. (1954). *Terminalia arjuna and its chemical constituents*. *J. Chem. Soc.*, 85, 399.
15. Dwivedi S. and Udupa N. (1989). *Terminalia arjuna*: pharmacognosy, phytochemistry, pharmacology and clinical use. A review. *Fitoterapia*, 60, 413-420.
16. Tripathi V.K. and Singh B. (1996). *Terminalia arjuna- its present status (a review)*. *Orient J.Chem*, 12, 1-16.
17. Cooper E.L. (2005). CAM, bioprospecting: the 21st century pyramid. Evid Based Compliment. *Alternat. Med.*, 2(2), 125-127.

18. Lister E. and Wilson P. (2001). Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Crop Research Institute, Lincoln, New Zealand, 235-239.

19. Tanrisever A. (1982). Investigations on relations between flavone contents and growth vigour in *Prunusavium*. *Journal of Ege University*, 19(2), 39-49.

20. Akillioglu M. (1994). Zeytin agaclarinda dogel fenolik bilesiklerin mevsimsel degisimi Cizerinde arastirmalar [Investigations on the seasonal changes of natural phenolic compounds in olive tree] (Doctoral dissertation, PhD thesis, Ege Univ. Fen Bilimleri Enst. Bahce Bitkileri Anabilim Dali, Izmir).

21. Ozeker E. (1994). Investigations on some biochemical changes in various organs of strawberry plants at different developmental stages. Doctorate Thesis. Ege Univ. Agric. Fac., Dept. of Horticulture, Bornova, Izmir. Turkey.