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The alkaloid content of poppy (*Papaver somniferum* L.) varieties in Turkey by their correlation and path coefficient relationships

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Abstract

The research was conducted with the aim of examining the alkaloids content of twelve poppies (*Papaver somniferum* L.) cultivars registered in Turkey by correlation and path analysis of relationships among alkaloids with capsule yield. The field experiments were set up according to a randomized complete block design with three replications in 2017-18 and 2018-19 under the semi-arid ecological conditions of Isparta, Turkey. The differences among the capsule yields and alkaloid contents of poppy varieties were statistically significant ($P \leq 0.01$) in both periods, and the capsule yields were determined to be between 253.4-556.2 kg ha^{-1} in the first year and 332.7-659.2 kg ha^{-1} in the second year. The morphine, codeine, oripavine, thebaine, papaverine, and noscapine contents of poppy cultivars varied between 0.46-1.84%, 0.08-0.30%, 0.009-0.057%, 0.031-0.093%, 0.002-0.047% and 0.017-0.094%, respectively. According to correlation analysis, the relationships between the morphine content with codeine (r=0.415*), oripavine (r=0.362*), and papaverine (r=0.624**) were significant and positive while noscapine (r=0.164^{ns}) and thebaine (r=0.043^{ns}) were insignificant and positive. In the path coefficient analysis, papaverine (p=0.631, 67.75%) had the highest direct effect on morphine content followed by thebaine, orpavine, and noscapine, respectively. Noscapine had the highest indirect positive effect on morphine content via papaverine while thebaine had negative and high indirect effects via papaverine. In the study, varieties that could be recommended for the semi-arid ecological conditions of Turkey were the TMO-1 and TMO-3 varieties due to higher capsule yields and the Ofis-1 variety due to the high alkaloid content.

Keywords: Poppy, Capsule yield, Morphine, Correlation, Path analysis

Introduction

The poppy (*Papaver somniferum* L.), belonging to the family *Papaveraceae*, is a valuable alkaloid plant. There are 36 species (58 taxa of which 15 are endemic) of poppies in Anatolia, and the *Papaver somniferum* L. species has traditionally been cultivated for thousands of years. Turkey is the world's important country in legal poppy cultivation and morphine supplier. Turkey has about 50% of the legal poppy planting area and supplies about 25% of legal morphine production. There are 15 poppy cultivars in the national varieties list of Turkey (SRCC, 2020). In Turkey, the poppy is cultivated over an area of 68 000 hectares with an annual production of 27 300 tons (TUIK, 2020), and the main cultivation regions are central Anatolia, the inner Aegean, and the western gateway zone. There are approximately 70000 registered poppy producers in 13 provinces of these regions.

Poppies contain approximately thirty different alkaloids with very high medicinal value (Prajapati et al., 2002); morphine, codeine, thebaine, noscapine, and papaverine are the main poppy alkaloids (Stranska et al., 2013). The most important of these is morphine ($C_{17}H_{19}O_3N$) due to its wide use as a powerful pain reliever and sedative, and its ratio in a dry capsule varies between 0.2-2.0%. The morphine, codeine, thebaine, noscapine, and papaverine content of Turkish poppy genotypes are 0.25-0.89%, 0.001-0.21%, 0.001-0.08%, 0.005-020%, and 0.004-021%, respectively (Arslan et al., 2009; Yazici and Yilmaz, 2017). Besides, poppy seeds contain approximately 40-55% oil and 20-30% protein, and seeds are used in Turkey in baked products such as donuts, bagels, and cakes (Kara, 2017).

The genetic, physiological, and morphological characteristics of poppy plants affect their production of capsule and seeds and their chemical content. In today's poppy breeding studies, the primary aim is to develop

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varieties with a high capsule yield and high morphine (or another opium alkaloid) content in the capsule. Capsule yield and alkaloid content of poppy are polygenic inheritances and are highly affected by the environment; therefore, it is crucial to know the direct or indirect relationships between traits to improve these characteristics. Generally, genetic and environmental factors that increased yield can have negative effects on quality (Baydar, 2020). Generally, capsule and seed yields of varieties with high morphine content are lower (Kara and Baydar, 2018) because poppies use a high level of photosynthetic assimilation and energy to synthesize more alkaloids. For this reason, breeders use correlation, regression, path, step-wise, and factor analysis to determine characteristics that showed a highly positive (direct and indirect) relationship with yield and quality for reliable selection criteria in searches (Baydar, 2020).

Plant breeding is used in correlation analysis to determine the relationship between yield and quality characteristics. However, correlation analysis only explains the relationships between independent characters, so it is necessary to use path analysis, which gives the relationship between the dependent variable and one or more independent variables. This research was conducted with aim of examining the relationships among alkaloids with the capsule yield and alkaloids contents of new poppy cultivars in Turkey by correlation and path analysis.

Materials and Methods

Experimental location

The field experiments were carried out during 2017-18 and 2018-19 under the semi-arid ecological conditions of Isparta, southwestern Anatolia, Turkey. The experiment conditions were typical of the continental climate with cold and snowy winters and dry and mild to temperate summers which are suitable for growing poppies with autumn production.

During the vegetative periods (from October to August) in 2017-18 and in 2018-19, there was an average temperature of 12.1 and 14.7°C, total precipitation of 431.0 and 574.3 mm, and average humidity of 63.4 and 63.1% (Table 1).

Table 1. Some climatic data of experimental area in growing seasons*

Climatic data	Years	Months											Mean total
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	
Mean temperature (°C)	2017-18	13.2	0.3	2.1	3.1	6.3	9.2	14.2	16.8	20.0	24.3	24.3	12.1
	2018-19	15.8	9.8	4.5	25	4.5	7.4	9.9	17.0	20.6	23.4	24.4	14.7
	Long term	12.9	7.4	3.5	1.9	2.9	6.2	10.7	15.6	20.2	23.6	23.2	11.6
Precipitation (mm)	2017-18	1.6	45.9	82.1	89.2	30.8	69.3	6.3	34.2	53.3	4.1	14.2	431.0
	2018-19	30.6	48.6	107.1	97.0	55.4	40.3	50.8	62.9	69.4	9.5	2.7	574.3
	Long term	38.0	46.3	84.9	72.2	64.7	54.2	56.0	51.4	29.8	14.6	10.5	522.6
Relative humidity (%)	2017-18	61.5	66.5	81.9	75.7	75.6	65.9	51.0	62.3	62.4	46.9	47.6	63.4
	2018-19	63.4	67.7	82.7	81.3	71.8	63.0	643	53.0	59.9	44.7	42.9	63.1
	Long term	62.0	68.5	74.7	73.2	70.2	65.4	61.3	57.4	51.1	45.4	46.3	61.4

*Meteorology Office Records

In the years 2017-18 and 2018-19, the soil at 60 cm was found to be sandy-loamy, low organic matter (1.92% and 1.70%, respectively), slightly alkaline (pH 7.3 and 7.5, respectively), and mid-limey (5.22% and 6.10% $CaCO_3$, respectively).

Seed material and experimental design

TMO-1, TMO-2, Ofis-95, and Ofis-96 with yellow seed; TMO-3, Ofis NM, Ofis-1, Ofis-2, and Ofis-3 with blue seed; Ofis-8 and Huseyinbey with white seed, and Ofis NP with seed pink were used for the genetic material in the research. The noscapine content of the Ofis NM and Ofis NP cultivars, the while morphine content of other cultivars are high. Generally, these cultivars are new varieties that have been registered in recent years in Turkey.

The field experiments were carried out in a randomized complete block design with three replicates in the period of the autumn sowing in 2017-18 and 2018-19. The seeds of each variety were sown at about a 0.5-1 cm depth, the spacing used was 0.40 x 0.15 m, and the plot length was 6 m with 6 rows on the 20th and 25th of October for the first and the second years, respectively. After emergence, one seedling was allowed to establish in each seedbed. The plants were non-irrigated at every growing stage. 100 kg nitrogen ha^{-1} (two equal doses at the sowing and at the 10-15 cm plant height stages) and 30 kg P_2O_5 ha^{-1} (all by sowing) fertilizers were applied to the form ammonium sulfate (21%) and triple super phosphate (43-46%), respectively (Aytekin and Onder, 2006). Plants were hoed at the time of the second fertilizing and were sprayed against aphids before flowering.

The capsules were manually harvested in the full ripeness period according to the maturity stage of the cultivars (moisture content of approximately 15-16%) from four rows in the center of each plot. After the harvest, capsules were dried at room temperature in the shade until reaching a moisture content of 10±0.10% (Kara, 2017). Then the seeds were separated from the capsules, and the capsule yield (kg ha^{-1}) was calculated by multiplying by 10000/plot sizes (m^2).

Alkaloids analysis

The alkaloid contents were analyzed only in the second year. Capsules of all varieties were ground after

drying at 70 ° C for 24 hours, and then the content (%) of morphine, codeine, oripavine, thebaine, papaverine, and noscapine was determined through HPLC-MS/MS analysis after the solvent extraction of poppy straw on dry matter at the laboratory of the Bolvadin Opium Alkaloids Factory (Karadavut and Arslan, 2006).

Statistical analysis

Capsule yield and alkaloid content data were analyzed according to the analysis of variance (ANOVA), and the significant differences between the group means were separated using the DUNCAN test. A matrix of simple correlation coefficients among the alkaloids was computed. The direct and indirect effects of traits on morphine content were made using path coefficient analysis. All analyzes were performed using the SPSS v.16.0 software (SPSS, Chicago, IL. USA).

Results and Discussion

Capsule yield

Capsule yield and the main alkaloid content of poppy cultivars are presented in Table 3. The mean capsule yield of poppy cultivars in the second year (543.8 kg ha^{-1}) was higher than the first year (392.3 kg ha^{-1}) (Table 2). These differences resulted from higher rainfall at the flowering and capsule growing periods (May and June) of plants in the second year compared with the first year (Table 1). In plants grown depending on natural precipitation (without extra irrigation), high rainfall significantly positively affects yield, because soluble nutrients elements are carried by water to growth points from the soil (Svobodova and Misa, 2004).

Differences between capsule yields of poppy cultivars were statistically significant ($P \leq 0.01$) in both years, varying between 253.4-556.2 kg ha^{-1} in the first year and 332.7-659.2 kg ha^{-1} in the second year. The highest capsule yields were obtained from the TMO-2, TMO-3, Ofis-3, Ofis-8, Ofis-95, Ofis-96, and Huseyinbey cultivars, while the lowest capsule yield was determined in the NP, Ofis NM, and Ofis-1 cultivars (Table 2). In a previous study, Kosar et al. (2014) reported that the capsule yield of poppies varied between 610-800 kg ha^{-1} in the Aksehir, 860-1170 kg ha^{-1} in the Bolvadin, 530-670 kg ha^{-1} in the Denizli, 610-740 kg ha^{-1} in the Eskisehir, and 760-1200 kg ha^{-1} in the Usak provinces of Turkey. It is true that our findings were lower than the results of Kosar et al. (2014), and these differences were probably due to climatic conditions, agronomic practices, genotype characteristics (root length, earliness or lateness, nutrient use efficiency) (Boydak and Kavurmaci, 2015; Kara, 2017).

Alkaloid content

The morphine, codeine, oripavine, thebaine, papaverine, and noscapine contents of poppies varied between 0.46-1.84%, 0.08-0.30%, 0.009-0.057%, 0.031-0.093%, 0.002-0.047%, and 0.017-0.094%, respectively. The morphine, codeine, oripavine, and papaverine content of Ofis-1, the thebaine content of Huseyinbey, and the noscapine content of the Ofis-2, Ofis-95, and Ofis-96 cultivars were higher. Generally, the lowest values for all alkaloids were determined to be in the TMO-3 cultivar (Table 2). Generally, varieties with high capsule yield (for example, TMO-2 and TMO-3) had low alkaloid content while varieties with low yield (for example, Ofis-1 and Ofis NM) had high alkaloid content. These varietal characteristics of varieties may vary depending on the spending on seeds or alkaloid synthesis of photosynthesis products during the capsule formation period. Dittbrenner et al. (2009) reported that the main alkaloid of the poppy is morphine, and other major alkaloids are noscapine, thebaine, oripavine, and papaverine. In the previous studies conducted in different regions of Turkey, the morphine, codeine, oripavine, thebaine, noscapine, and papaverine contents of the poppy varied between 0.593-1.453%, 0.000-0.237%, 0.000-0.104%, 0.000-0.523%, 0.000-1.793%, and 0.000-0.350%, respectively, in Ankara conditions (Ozgen et al., 2017); and 0.15-0.60%, 0.001-0.21%, 0-0.01%, 0.001-0.08%, 0.005-0.20%, and 0.004-0.21%, respectively, in Tokat conditions (Yazici and Yilmaz, 2017). The morphine, codeine, thebaine, papaverine and noscapine ranged from 0.110-1.140%, 0.005-0.27%, 0.005-0.134%, 0.001-0.440% and 0.006-0.418%, respectively in Afyonkarahisar conditions (Gumuscu et al., 2008). In our study, the morphine, codeine, oripavine, and papaverine content of the Ofis-1 variety and thebaine content of the Huseyinbey variety were significantly higher than in previous studies. The results showed that the alkaloid's content changed according to the varieties and climatic conditions. Similarly, Franz (1983) explained that the poppy alkaloid's contents were under the influence of genetic and environmental factors.

Table 2. Capsule yield and major alkaloid contents of poppy cultivars

Varieties	Capsule yield (kg ha^{-1})		Alkaloids (%)$^{+}$					
			Morphine	Codeine	Oripavine	Thebaine	Papaverine	Noscapine
	2017-18	2018-19						
TMO-1	367.3 d	564.2 b	0.52 de	0.16 bcd	0.014 fg	0.056 de	0.002 e	0.035 cd
TMO-2	556.2 a	589.3ab	0.46 e	0.17 bc	0.018 efg	0.053 e	0.003 de	0.053 bc
TMO-3	429.1cd	659.2 a	0.49 e	0.12 de	0.009 g	0.070 bc	0.004 de	0.057 bc
Ofis-1	272.4 e	338.1de	1.84 a	0.30 a	0.057 a	0.042 f	0.047 a	0.056 bc
Ofis-2	381.5d	435.7 c	1.19 b	0.15 bcd	0.013 g	0.080 b	0.035 b	0.086 a
Ofis-3	377.2 d	639.2ab	0.79 cde	0.14 cd	0.013 g	0.031 f	0.046 a	0.076 ab
Ofis-8	364.4 d	646.5ab	0.90 bcd	0.15 bcd	0.024 cde	0.071 bc	0.005 cd	0.017 d
Ofis-95	471.6bc	631.6ab	0.70 cde	0.13 de	0.035 b	0.032 f	0.003 de	0.087 a
Ofis-96	429.1cd	632.4ab	0.61 cde	0.08 e	0.033 bc	0.065 cd	0.005 de	0.094 a
Ofis NM	271.6 e	422.8cd	0.95 bc	0.28 a	0.029 bcd	0.048 e	0.005 cd	0.070 ab
Ofis NP	253.4 e	332.7 e	0.78 cde	0.13 cde	0.023 def	0.058 de	0.009 c	0.060 bc
Huseyinbey	534.7ab	618.1ab	0.54 de	0.19 b	0.010 g	0.093 a	0.003 de	0.038 cd
Year $_{Mean}$	392.3 B**	542.8 A	0.81	0.16	0.023	0.058	0.014	0.061
Mean square	288.8	461.9	0.296	0.123	0.057	0.012	0.093	0.162
F value $_{Cultivar}$	32.07**	31.48**	10.81**	27.16**	36.41**	54.73**	368.74**	12.92**
F value $_{Year\ x\ cult.}$		2.84^{ns}						
CV (%)	7.65	7.06	11.08	8.78	7.15	8.31	6.37	8.43

**: significant at P<0.01 probability levels, ns: non-significant

$^{+}$Alkaloids contents of poppy belongs to in 2018-19.

Means in the same columns followed by the same letters are not significantly different as statistically

Correlation and path coefficient analysis

The results of correlation analysis among the alkaloids of the poppy cultivars are shown in Table 3. According to the results of the correlation analysis, while there were significant and positive correlations between morphine with codeine (r=0.415*), oripavine (r=0.362*), and papaverine (r=0.624**), an insignificant and positive correlation was determined between noscapine (r=-0.164^{ns}) and thebaine (r=0.043^{ns}) in the poppies (Table 3). Significant and negative correlations were determined between oripavine and thebaine (r=-0.532**) while there were positive and insignificant correlations between papaverine (r=0.264^{ns}) and noscapine (r=0.207^{ns}). Significant and negative correlations were determined between thebaine with papaverine (r=-0.374*) while there was a non-significant correlation of thebaine with noscapine (r=0.228^{ns}). Insignificant correlations were determined between papaverine and noscapine (r=0.280^{ns}) in the poppy (Table 3).

Table 3. Correlation coefficient matrix of alkaloids

Alkaloids	Morphine	X_1	X_2	X_3	X_4	X_5
Codeine (X_1)	0.415*	1.000				
Oripavine (X_2)	0.362*	0.435**	1.000			
Thebaine (X_3)	0.043^{ns}	-0.204^{ns}	-0.532**	1.000		
Papaverine (X_4)	0.624**	0.317^{ns}	0.264^{ns}	-0.374*	1.000	
Noscapine (X_5)	0.164^{ns}	0.105^{ns}	0.207^{ns}	0.228^{ns}	0.280^{ns}	1.000

*, **: significant at P<0.05 and P<0.01 probability levels, respectively, ns: non-significant

In our research, morphine as a dependent variable and codeine, oripavine, thebaine, papaverine, and noscapine as determinative variables were used for the path coefficient analysis. The direct and indirect contributions to the morphine of the major alkaloids in the poppy cultivars are given in Table 4. The direct effects on morphine of the major alkaloids were positive. The highest positive direct effect on morphine content was papaverine (p=0.631, 67.75%) followed by thebaine (p=0.413, 47.54%), oripavine (p=0.343, 42.83%), codeine (p=0.152, 25.86%), and noscapine (p=0.027, 7.01%), respectively (Table 4). Papaverine had the highest indirect positive contribution to morphine content via noscapine (p=0.176, 45.82%) and codeine (p=0.200, 33.97%) (Table 4). Thebaine had an indirect negative effect on morphine content via all examined alkaloids (Table 4). These results indicated that the direct and indirect effect on morphine content of papaverine was higher than the other alkaloids. Bajpai et al (2001) stated that there was a significant and positive relationship between morphine and codeine. Shukla et al (2003) reported negative relationships between morphine and other poppy alkaloids. Yadav et al. (2006) reported that bilateral relationships between morphine, codeine, thebaine, and papaverine were positive while there was a negative relationship between morphine and papaverine; the same studies reported that alkaloids can transform into each other depending on the alkaloid synthesis pathway. Prajapati et al. (2002) reported that the morphine alkaloid was synthesized from codeine rather than oripavine, and there was a positive relationship between morphine and codeine. Psenak (1998) explained that oripavine alkaloid was the final product in the synthesis of morphine in some poppy species but that it is transformed into morphine via thebaine, codeinone, and codeine in *Papaver somniferum*. Besides, the same researcher reported that codeine was synthesized as a result of the reduction of

codeinone, and oripavine transformed into morphine by linking hydrogen to its chemical structure. Dittbrenner et al. (2009) stated that codeine was the pioneer of morphine synthesis and that sometimes the morphine pathway was blocked during enzymatic activity, and the conversion from codeine to morphine stops. We can report that the results of our study were similar to the findings of previous researchers.

Table 4. Path coefficient (direct and indirect effects) among alkaloids

Alkaloids	Direct effects	Indirect effects				
	Morphine	X_1	X_2	X_3	X_4	X_5
Codeine (X_1)	0.152	-	0.066	-0.031	0.048	-0.016
	25.86		8.27	3.57	5.17	4.27
Oripavine (X_2)	0.343	0.149	-	-0.182	0.090	0.071
	42.83	25.38		20.99	9.70	18.42
Thebaine (X_3)	0.413	-0.08	-0.219	-	-0.154	-0.094
	47.54	14.28	27.40		16.56	24.40
Papaverine (X_4)	0.631	0.200	0.166	-0.236	-	0.176
	67.75	33.97	20.77	27.17		45.82
Noscapine (X_5)	0.027	-0.002	0.005	-0.006	0.007	-
	7.01	0.49	0.69	0.70	0.81	

The first lines are path coefficient (pc) and the second lines are path percentage (%)

Conclusions

In this research, the highest capsule yield was obtained from the TMO-2 and TMO-3 varieties. Ofis-1 had the highest morphine, codeine, oripavine, and papaverine content while the thebaine content of Huseyinbey and the noscapine content of Ofis-2, Ofis-95, and Ofis-96 were higher.

There were significant and positive correlations between morphine content with codeine, oripavine, and papaverine while bilateral relations between alkaloids showed a difference. Papaverine had the highest direct and positive effect on morphine followed by thebaine, oripavine, and noscapine, respectively. Noscapine had the highest indirect positive effect on morphine via papaverine. Thus, relationships among alkaloids should be taken into consideration in selection breeding with high alkaloid content because the average morphine content in dry capsules of local varieties, which are widely cultivated in Turkey, is around 0.4%. However, the contents of morphine and its derivatives are around 1-2% in some countries; therefore, the breeding of varieties with both high capsule yield and high morphine content in Turkey should be studied.

Compliance with Ethical Standards

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Designed of the experiment and its conducted (Kara, N), prepared of plant materials and collected experiment data (Kara, N), performed of CS-MS analyses (Kara N), wrote the manuscript (Kara, N and Baydar, H). Both authors read and approved the final manuscript.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

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