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Determining Relationships Amongst Morphine, Capsule and Oil Yield Using Path Coefficient Analysis in Poppy (*Papaver somniferum* L.)

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In this paper, morphine content, oil content, seed yield, capsule yield, oil yield and morphine yield were determined for four poppy cultivar, grown according to the randomized complete blocks in a split-plot design with 4 replications in Central Anatolia. Statistically significant variations were found by ANOVA amongst the genotypes for all the variables examined. The morphine, capsule and oil yield of poppy cultivars vary between, Kocatepe-96 4.12 kg ha^{-1}, 805.6 kg ha^{-1}, 42.88 kg ha^{-1}; Karahisar-96 5.64 kg ha^{-1}, 863.6 kg ha^{-1}, 55.61 kg ha^{-1}, respectively. Correlations amongst the variables as well as their direct and indirect effects on morphine, capsule and oil yield were also calculated using the correlation and path coefficients analyses, respectively. Positive and significant relationships were found statistically between (a) morphine yield and morphine content, seed yield, capsule yield, oil yield; (b) between seed yield and capsule yield, oil yield and (c) between capsule yield and oil yield. Path coefficient analyses indicated that morphine content (77.86 %), capsule yield (83.73 %), seed yield (10.30 %) and oil yield (1.89 %), had a positive direct effect on morphine yield.

Key Words: Poppy, Morphine, Capsule, Oil, Correlation coefficient, Path coefficient.

INTRODUCTION

Poppy, *Papaver somniferum* L., is an annual medicinal herb native to Southeastern Europe and Western Asia. Also known as *Opium poppy*, the species is cultivated extensively in many countries, including Iran, Turkey, Holland, Poland, Romania, Czech Republic, Slovakia, Yugoslavia, India, Canada and many Asian and Central and South American countries. There is about 99.431 ha poppy sowing area in Turkey[1]. Turkey is the most important country for poppy capsule and seed production. Grown in Turkey and Holland, these small to medium size oval shaped poppy pods are light green and golden brown in colour. The Turkish poppies are known for their small size.

Poppy is a dual purpose crop; its capsules are the source of edible seeds and the life-saving alkaloid drugs, morphine and codeine; the former is a preferred analgesic for cancer patients and the latter a cough depressant[2]. Poppy seeds are used as a condiment with baked goods and pastries for their nutty odour and flavour. Poppy oil is widely used as an edible cooking oil. The oil is also used in the manufacture of paints, varnishes and soaps. Opium is used in the production of morphine, codeine, other alkaloids and deodorized forms of opium. Morphine is the raw material from which heroin is obtained. Poppy plants are important as ornamental plants in flower gardens. Opium and alkaloids are extracted from the dried milky sap of the immature seed capsules. Morphine and codeine are two of the most well-known opium alkaloids. The mature seeds are used predominantly in poppy cakes but also in smaller amounts on rolls and bagels. Because of their high oil content, edible oil is produced from the seeds, too. Although the seeds may also contain alkaloids, they only occur naturally in traces. Hence, edible poppy seeds are only likely to contain the smallest amounts of morphine. The increasing demand for poppy alkaloids is a consequence of the widening medical application of morphine and morphine related compounds[3]. The consumption of morphine since the second half of the 1980s has increased steadily[4]. However, to limit drug abuse in European countries in accordance with administrative regulations, a new strategy was accepted by governments and commercial producers interested in the large-scale cultivation of poppy. Consequently, the creation of new cultivars with an especially high alkaloid content (15-25 mg/100 g morphine) and of alkaloid-free cultivars for seed production (accumulating less than 0.2 mg/100 g morphine in capsules) was intensified[5,6]. Poppy seeds are the ripe seeds harvested from the capsules of *Papaver somniferum* L. (opium poppy, family: *Papaveraceae*). Owing to their content of fatty oil (40-60 %) and protein (15-24 %), the seeds have been a popular source of food (for example in poppy-seed cake, desserts *etc.*). In poppy seeds, in contrast to other parts of the plant, the alkaloid-rich latex (milky juice) is not found. Therefore, poppy seeds contain the alkaloids only in traces according to the scientific literature and have not been included in regulations under the Narcotics Act. Partially the same plants from which the seeds derive are used to obtain opium (dried latex from unripe capsules) or opium alkaloids. In addition to the primary alkaloid, morphine, there are secondary alkaloids such as codeine, thebaine, noscapine (formerly referred to as narcotine) and papaverine found in opium[7].

Correlation coefficients generally show relationships among independent characteristics and degree of linear relation between these characteristics. It is not sufficient to describe this relationship when the causal relationship among characteristics is needed[8]. Path analysis is used when we wish to

know causes. In other words, path analysis is used when we desire to determine the amount of direct and indirect effect of causal components on the effect component[9]. The advantage of path analysis is that it permits the partitioning of the correlation coefficient into its components, one component being the path coefficient that measures direct effect of a predictor variable upon its response variable; the second component being the indirect effects of a predictor variable on the response variable through another predictor variable[10].

The aim of this research was to determine relationships among morphine content and oil content, seed yield, morphine yield, oil yield and capsule yield which characteristics directly affected and how much morphine, oil and capsule yield variation was apparent in poppy genotypes.

EXPERIMENTAL

This research was carried out at the experimental field of the Afyon Kocatepe Agricultural Research Institue, Afyon, Turkey. The present study was conducted on clay-loam soils and soil properties of the research area are shown in Table-1. Climatic data related to the research area shown in Table-2. The research was conducted with four poppy cultivars named Ankara-94 (white seed colour) and Karahisar-96 (yellow seed colour);

TABLE-1
SOIL PROPERTIES OF THE RESEARCH LOCATION

Organic matter (%)	1.90	Texture	Clay-loam
$CaCO_3$ level (%)	16.50	Available P_2O_5 (ppm)	6.87
pH	7.61	Available K_2O (ppm)	80.72
E. conduct. (mmhos/cm)	0.03	Zn (ppm)	0.56
Saturation	62.50 Cl	B (ppm)	2.84

TABLE-2
CLIMATIC DATA OF THE RESEARCH LOCATION

Months	Rainfall (mm)	Temperature (ºC)	Relative humidity (%)
October 2002	34.3	12.7	57.7
November	14.0	7.6	63.4
December	48.9	-1.4	70.5
January 2003	23.9	5.5	69.1
February	98.1	-1.9	77.6
March	29.4	2.3	67.4
April	56.1	9.4	67.2
May	58.5	17.2	58.6
June	14.4	20.5	52.2
July	0.0	22.5	52.1
Total	377.6	–	–
Mean	–	9.4	63.5

Kocatepe-96 (white seed colour) and Afyon Kalesi-95 (yellow seed colour) (registered by Variety Registration and Certification Centre, Republic of Turkey). The field experiment was established as a randomized complete block in a split-plot arrangement with four replications. Poppy cultivars was grown different B levels (0, 1, 3, 9 and 36 kg ha^{-1}) conditions. Statistical analysis for examined characters were done on average datas of different B levels applications. The previous crop was wheat. Sowing was made in 3 m long rows with 35 × 15 cm row spacings on 15 October 2002. 50 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} nitrogen applied to each plot. Routine management practices were followed. Plots were harvested in the stage of full ripeness on 17 July 2003 from an internal area of 3.5 m^2, after removing two outer rows at each plot. This area was designated as the harvest area. The following parameters were evaluated seed, oil and capsule yield per hectar, oil content of seed, content of morphine in ripe capsules. The alkaloid morphine was determined by Hewlett-Packard High Performance Liquid Chromatography in Bolvadin Alkoloid Fabric Laboratories. Oil content was determined according to the Soxhlet methods.

Statistical analysis: Experimental data were subjected randomized complete block in a split-plot model of ANOVA and the F-test applied to examine the statistical significance of different amongst the varieties. All significant main effects interactions were considered. Analysis of variance, Duncan's multiple range test, coefficients of correlation analysis and path coefficient analysis were performed using the computerized statistical program TARIST obtained from the Faculty of Agriculture, Ege University, Izmir, Turkey. The effects of examined characters on morphine, capsule and oil yield were determined by using the path coefficient technique separately for the four poppy cultivars. In order to determine the relationships between examined characteristics and on morphine, capsule and oil yield, correlation coefficients were first calculated. Path coefficients were then calculated to understand the direct character effects on morphine, capsule and oil yield. The path coefficient is known as a standardized partial-regression coefficient and seperates the direct and indirect effects of a correlation coefficient. Hence, path analysis plays an important role in determining the degree of relationship between morphine yield and quality components.

RESULTS AND DISCUSSION

There were statistically significant ($p < 0.01$) differences amongst the cultivars with respect to oil content, morphine content, seed yield, morphine yield and oil yield (Table-3). Large variations resulted in statistically different groups with respect to morphine content (A. Kalesi, 0.51 %; Karahisar-96, 0.65 %), oil content (Ankara-94, 49.90 %; Karahisar-96, 51.81

%), seed yield (Kocatepe-96, 829.6 kg ha^{-1}; Karahisar-96, 1076.5 kg ha^{-1}), morphine yield (Kocatepe- 96, 4.12 kg ha^{-1}; Karahisar-96, 5.64 kg ha^{-1}), oil yield (Kocatepe-96, 42.88 kg ha^{-1}; Karahisar-96, 55.61 kg ha^{-1}) (Table-3).

TABLE-3
MEAN DATA AND STATISTICAL GROUPS OF VARIOUS POPPY CULTIVARS WITH RESPECT TO VARIABLES ANALYZED

Cultivar	Morphine content (%)	Oil content (%)	Seed yield (kg ha^{-1})	Capsule yield (kg ha^{-1})	Morphine yield (kg ha^{-1})	Oil yield (kg ha^{-1})
Ankara-94	0.59 ab‡	49.90 b‡	895.4 bc‡	806.0‡	4.85 b‡	44.58 bc‡
Kocatepe-96	0.52 bc	51.67 a	829.6 c	805.6	4.12 c	42.88 c
A. Kalesi	0.51 c	51.67 a	977.7 ab	857.9	4.39 bc	50.36 ab
Karahisar-96	0.65 a	51.81 a	1076.5 a	863.6	5.64 a	55.61 a
Mean	0.57	51.26	944.8	833.3	4.75	48.36

‡Within columns, means followed by the same letter are not significantly different by ANOVA protected Duncan's multiple range test ($p < 0.01$).

Gümüscü and Arslan[11] indicated that average seed yield, capsule yield, morphine ratio and morphine yield varied between 449-1281 kg ha^{-1}, 614-697 kg ha^{-1}, 0.57-1.40 % and 4.68-8.52 kg ha^{-1}, respectively for winter sowing in select desirable poppy lines introduced from different regions. Bajpai *et al.*[12] estimated that seed and capsule yield of poppy genotypes vary between 419-732 and 1.819-3.075 kg ha^{-1}, respectively in Indian conditions. Erdurmus[13], Novak and Strakova[14] and Karadavut[15] reported that the morphine content of poppy genotypes vary between 0.22 and 1.22 % from genotype to genotype. Variations amongst the cultivars in examined characters can be attributed to varying types of poppy, the type of vegetation cycle and geographical origin as well as environmental factors.

Correlation and path analyses: Simple correlation coefficients calculated among examined characteristics are shown in Table-4. Positive and

TABLE-4
CORRELATION COEFFICIENTS BETWEEN QUALITY CHARACTERS AND SEED YIELD IN VARIOUS POPPY CULTIVARS

Variable	Morphine yield	Morphine content	Oil content	Seed yield	Capsule yield	Oil yield
Morphine yield	-	0.491‡	-0.104	0.919‡	0.906‡	0.891‡
Morphine content		-	-0.195	0.204	0.107	0.192
Oil content			-	-0.011	-0.015	0.038
Seed yield				-	0.964‡	0.980‡
Capsule yield					-	0.939‡
Oil yield						-

‡Indicates significance at $p < 0.01$.

significant relationships were found statistically between morphine yield and morphine content, seed yield, capsule yield, oil yield; between seed yield and capsule yield, oil yield; between capsule yield and oil yield.

Correlation coefficients calculated between morphine, capsule, oil yield and other variables and path coefficient analysis revealing direct and indirect effects of variables on morphine, capsule and oil yield, are given in Tables 5-7, respectively.

Morphine content and capsule yield had high direct effects on morphine yield. A significant positive relation was found between the morphine yield and capsule yield, of which 83.73 % was due to a direct effect and 16.27 % to an indirect effect, especially through the seed yield. Similarly, a significant positive relation was found between morphine yield and morphine content, with a direct effect of 77.86 % and an indirect effect of 22.14 %, of which 16.85 % was mainly through capsule yield. The direct effect of seed yield and oil yield was positive and statistically significant (r = 0.919‡ and r = 0.891‡, respectively). But seed and oil yield had low direct effects on morphine yield. The ratio of the direct effect of seed yield on morphine yield is 10.30 %. In other words, 89.70 % of the variation in morphine yield comes from the indirect effects of seed yield on morphine yield. Indirect effect of seed yield on morphine yield of which 79.58 % was due to especially through capsule yield. Similar results determined for oil yield characters (Table-5).

The consumption of morphine has increased steadily per year. However, to limit drug abuse in European countries in accordance with administrative regulations, a new strategy was accepted by governments and commercial producers interested in the large-scale cultivation of poppy. Consequently, the creation of new cultivars with an especially high alkaloid content (15-25 mg/100 g morphine) and of alkaloid-free cultivars for seed production (accumulating less than 0.2 mg/100 g morphine in capsules) was intensified[5,6]. The morphine production could be influenced under Turkey conditions by the morphine content and capsule yield of cultivars, climate and soil conditions.

Morphine content and morphine yield had high direct effects on capsule yield. The direct effect of morphine yield on capsule yield was positive and statistically significant (r = 0.906‡) and the ratio of the direct of morphine yield on capsule yield is 62.54 %. A non-significant positive relation (r = 0.107) was found between the morphine content and capsule yield, of which 42.65 % was due to a direct effect and 57.35 % to an indirect effect, especially through morphine yield (48.71 %). Positive significant relationship between capsule yield and seed yield (r = 0.964‡) but seed yield had low direct effects on capsule yield. The ratio of the direct effect of seed yield on capsule yield is 26.80 %. Indirect effect of seed yield on capsule yield was due to especially through morphine yield (64.32 %; Table-6).

TABLE-5
PATH COEFFICIENT ANALYSIS BETWEEN MORPHINE YIELD AND OTHER VARIABLES EXAMINED IN DIFFERENT POPPY CULTIVARS

Variable	Coefficient of correlation[1]	Indirect effects											
		Direct effects		Morphine content		Oil content		Seed yield		Capsule yield		Oil yield	
		p[2]	%	p	%	p	%	p	%	p	%	p	%
Morphine content	0.491**	0.387	77.86	-	-	0.0020	0.57	0.020	4.02	0.083	16.85	-0.0030	0.67
Oil content	-0.104	-0.014	14.10	-0.075	72.77	-	-	-0.001	1.00	-0.011	11.46	-0.0007	0.64
Seed yield	0.919**	0.098	10.30	0.078	8.27	0.0002	0.016	-	-	0.758	79.58	-0.0170	1.81
Capsule yield	0.906**	0.786	83.73	0.041	4.39	0.0002	0.023	0.094	10.08	-	-	-0.0160	1.76
Oil yield	0.891**	-0.017	1.89	0.074	8.00	-0.0006	0.060	0.096	10.37	0.738	79.65	-	-

[1]For coefficient of correlation, see also Table 4; [2] p, path coefficient; **Indicates significance at $p < 0.01$.

TABLE-6
PATH COEFFICIENT ANALYSIS BETWEEN CAPSULE YIELD AND OTHER VARIABLES EXAMINED IN DIFFERENT POPPY CULTIVARS

Variable	Coefficient of correlation[1]	Indirect effects											
		Direct effects		Morphine content		Oil content		Seed yield		Morphine yield		Oil yield	
		p[2]	%	p	%	p	%	p	%	p	%	p	%
Morphine content	0.107	-0.352	42.65	-	-	-0.0010	0.130	0.063	7.730	0.402	48.71	-0.006	0.760
Oil content	-0.015	0.005	3.58	0.068	41.82	-	-	-0.003	2.035	-0.085	51.80	-0.001	0.750
Seed yield	0.964**	0.314	26.80	-0.071	6.12	-0.0001	0.005	-	-	0.754	64.32	-0.032	2.740
Morphine yield	0.906**	0.820	62.54	-0.173	13.18	-0.0006	0.046	0.288	21.980	-	-	-0.029	2.229
Oil yield	0.939**	-0.032	2.87	-0.067	5.93	0.0002	0.010	0.307	26.990	0.731	64.17	-	-

[1]For coefficient of correlation, see also Table 4; [2] p, path coefficient; **Indicates significance at $p < 0.01$.

TABLE-7
PATH COEFFICIENT ANALYSIS BETWEEN OIL YIELD AND OTHER VARIABLES EXAMINED IN DIFFERENT POPPY CULTIVARS

Variable	Coefficient of correlation[1]	Indirect effects											
		Direct effects		Morphine content		Oil content		Seed yield		Capsule yield		Morphine yield	
		p[2]	%	p	%	p	%	p	%	p	%	p	%
Morphine content	0.192	0.006	2.43	-	-	-0.0090	3.570	0.2160	85.40	-0.0060	2.39	-0.015	6.18
Oil content	0.038	0.046	73.48	-0.001	1.910	-	-	-0.0110	17.97	0.0009	1.36	0.003	5.25
Seed yield	0.980**	1.063	92.51	0.001	0.109	-0.0005	0.043	-	-	-0.0540	4.78	-0.029	2.55
Capsule yield	0.939**	-0.057	5.12	0.0007	0.059	-0.0007	0.063	1.0252	92.15	-	-	-0.028	2.60
Morphine yield	0.891**	-0.031	2.98	0.003	0.280	-0.0040	0.450	0.9760	91.44	-0.0510	4.83	-	-

[1]For coefficient of correlation, see also Table 4; [2] p, path coefficient; **Indicates significance at $p < 0.01$.

Poppy capsule production was important drug industry. Generally, most of country that cultivated poppy were utilized dried capsule for alkaloid production. A latex containing several important alkaloids is obtained from immature seed capsules 1-3 weeks after flowering. Incisions are made in the walls of the green seed pods and the milky exudation is collected and dried. Opium and the isoquinoline alkaloids morphine, codeine, noscapine, papaverine and thebaine are isolated from the dried material. The poppy seeds and fixed oil that can be expressed from the seed are not narcotic, because they develop after the capsule has lost the opium-yielding potential. Total yield of alkaloids is dependent on light, temperature, the plant species, geographical origin and the time of harvest. According to Bernáth and Tetenyi[16] the morphine accumulation in dry matter a condition-system considered as depending upon annual climatic conditions between years (*e.g.*, temperature, humidity *etc.*). In Hungary, Petheö *et al.*[17] 10 poppy cultivars have been registered with different alkaloid contents of dry capsule. Their idea was - in conformation with international efforts observed in poppy breeding - to select plant material of winter type with low-, high- or special alkaloid content. Morphine content between 1.5 and 4.5 % with individual values of 0.2-10.0 %. The relatively high individual variability found in the alkaloid content of populations provides an excellent basis for the further selection. In the production of alkaloids the role of the cultivated area was significant. On the one hand, the greater space appreciably increased production per plant due to the higher capsule number and capsule weight. On the other hand, the favourable effect of more space did not occur when alkaloid production was related to unit area. A larger plant number of 20 × 20 cm area both in the autumn and spring cultivars accumulated more morphine[16] (from 20 to 100 %) per m^2.

Seed yield and oil content had high direct effects on oil yield. Significant positive relation was found between the oil yield and seed yield (r = 0.980‡), of which 92.51 % was due to a direct effect and 7.49 % to an indirect effect, especially capsule yield. The ratio of the direct effect of oil content on oil yield is 73.48 %. Positive significant relationship between oil yield and capsule yield (r = 0.939‡) but capsule yield had low direct effects on oil yield. The ratio of the direct effect of capsule yield on oil yield is 5.12 %. Indirect effect of capsule yield on oil yield was due to especially through seed yield (92.15 %; Table-7). Morphine content and morphine yield had low direct effects on oil yield (2.43 and 2.98 %, respectively).

Poppy seed is generally recognized as safe for human consumption as a spice or a natural flavouring. Opium poppy seeds have a high lipid content (50 % of the seed dry weight[18]). The non-polar lipids, particularly triacylglycerols, constituted a major portion (86 %) of the total lipids. The deposition of triacylglycerols and hence oil followed a sigmoidal pattern,

similar to the increase in seed dry weight. The active period of their synthesis lies between 15 and 20 d after flowering. The relative percentages of polar lipids (phospho- and glycolipids), sterols and free fatty acids in the oil declined with seed maturation. However, most types of lipids in reased in net terms, when measured on per seed basis. Though palmitic, oleic and linoleic were the major fatty acids at all the stages of the seed development, there was clear predominance of linoleic acid. The proportion of linoleic acid increased tremendously with the deposition of triacylglycerols and was negatively correlated with linoleic acid. The changes in the fatty acid make up were quite marked in the early developmental stages than at the later stages of the seed development[19].

Conclusion

The aim of poppy agronomy were combined high yield potential for seed, morphine and oil. Studies on alkaloid levels in edible poppy seeds have, however, revealed that the levels vary markedly and have increased overall in recent years. Types of poppy, harvesting time and geographical origin could all influence the alkaloid levels. The results of the study showed that selection efforts to increase for morphine yield of poppy genotypes studied should focus on morphine content, capsule yield, which have direct effects on morphine yield and positive correlated with it. However, oil content and seed yield which have important direct effects, should also be taken into account during selection of better oil yielding genotypes. Morphine content, oil content, seed yield, morphine yield and oil yield were significantly affected by poppy cultivars.

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