

Original article

Comparison of Yield and Yield Characteristics in Different Rice Hybrids and Investigation of Their Inheritance

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Abstract

The objective of this study was to determine the heterosis and heterobeltiosis values of yield components and some important traits in rice (*Oryza sativa* L.) and to estimate the narrow-sense heritability of these traits. For this purpose, in 2020, the F1 hybrid combinations of Halilbey x Aga, Halilbey x Gemici, and Halilbey x Vasco, obtained from the parental lines (Halilbey, Aga, Gemici, Vasco) used in hybridization, were cultivated in the Gönen district of Balıkesir. The transplanting method was used for planting. In the transplanting, one seedling was planted per each hill at a planting density of 25x25 cm. In this study, observations and measurements were made for the following characteristics: the flowering days, maturity days, plant height, panicle length, the number of panicles per plant, the number of grains per panicle, thousand-grain weight, and grain yield per plant. In the heterosis and heterobeltiosis evaluations, the highest heterosis and heterobeltiosis values were determined for panicle number per plant and rice yield per plant. High levels of narrow-sense heritability were estimated for the number of panicle, rice yield per plant, and maturity days, while moderate levels were determined for the plant height and thousand-grain weight, and low levels for flowering days, panicle length, and number of grains per panicle. The high levels of heterosis and heterobeltiosis observed in rice yield are attributed to the high levels of heterosis and heterobeltiosis in the number of panicle per plant. Additionally, this trait exhibits a high degree of narrow-sense heritability. Utilizing this trait in hybrid rice breeding could lead to increased yield. Therefore, this trait may be used as a selection criteria in heterosis breeding in rice. At the same time, flowering day and panicle length should be taken into account in the selection.

Keywords: Rice (*Oryza Sativa* L.), Heterosis, Heterobeltiosis, Narrow Sense of Heritability, Rice Breeding.

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INTRODUCTION

There are 24 species belonging to the *Oryza* genus of the *Gramineae* family. Two of these are the *O. sativa* and *O. glaberrima* species, which are cultivated species. The remaining 22 species are wild species that are widely distributed throughout the world. Rice is the world's most important food product and is the primary food source for more than one-third of the world's population. Over 90% of the world's rice is grown and consumed in Asia, where 60% of the world's population lives. Rice accounts for 35% to 60% of the calories consumed by 3 billion Asians (Khush, 1997).

Rice (*Oryza sativa* L.) is cultivated in over 100 countries across the world, covering an area of 168 million hectares. The global rice production volume is 800 million tons (FAO, 2023). More than 90% of rice production conducted and consumed in Asian countries, meeting three-quarters of the daily calorie needs of 500 million Asians. To meet the world's population needs, rice production must increase by 28% by 2035 and by 22% by 2050 (IRRI, 2020). The major rice-producing countries in the world are China, India, Thailand, Vietnam, Bangladesh, Indonesia, Burma, Pakistan, Brazil, the United States, etc.

In Turkey, rice is cultivated on an area of 128,000 hectares, with a production of approximately 1 million tons. 70% of rice is produced in the Thrace-Marmara region, 25% in the Black Sea region, and the remaining 5% in the other regions. Turkey's average rice yield per hectare is around 8 ton. The most important rice-growing provinces are Edirne, Samsun, Balıkesir, Çanakkale, Çorum, Bursa, Tekirdağ, Çankırı, Sinop, and Kastamonu (TÜİK, 2025).

Rice is a highly self-pollinating plant with a very low rate of cross-pollination. This is also the main reason why heterosis is not commercially utilized in rice. This issue has been resolved through the use of cytoplasmic male sterility. Hybrid rice breeding in China was initiated in 1964 by L.P. Yuan. In 1972, the male sterile line and its maintainer line, Er-jiu-nan-1, were developed. After identifying the restorer line, the three-line system was completed, and the first commercial hybrid variety, Nanyou 2, was developed. In 1976, hybrid rice became commercially available and began to spread. Generally, hybrid varieties yield 20-30% higher than conventional varieties. In China, when hybrid rice was first used commercially in production in 1979, the average rice yield was 2.7 ton per hectare. This average yield became to 4.4 ton per hectare in 1985 and reached 6.6 ton per hectare by 1990 (Ma and Yuan, 2015).

The price of hybrid seeds is ten times higher than that of standard varieties. In countries where hybrid rice is produced, transplanting method is practiced per planting. This requires the use of 40-50 kg of seeds per hectare. In Turkey, rice planting carried out using direct seeding method. For this, 200 kg of seeds need per hectare (Surek, 2002).

In heterozygous plants obtained by crossing self-pollinated lines, a yield increase called hybrid vigor is observed. The phenomenon of hybrid vigor was first discovered by Koelreuters in the 1760s (Hayes et al., 1995). Shull, who studied the yield and development of self-pollinated lines in maize crop starting in 1909, first introduced the concept of “heterosis” in 1914. Heterosis is the term given to the phenomenon observed in F₁ hybrid generation plants, where they exhibit higher strength, vigor, productivity, growth rate, and resistance to diseases, pests, and adverse environmental conditions compared to the average of their parental lines (Hayes et al., 1995). Heterobeltiosis, on the other hand, refers to the superiority exhibited relative to the higher-yielding parent.

The first heterosis in rice determined by Jones (1926) in California. The researcher detected 69% heterobeltiosis in rice yield. In rice yield, Carnahan et al. (1972) reported heterobeltiosis ranging from -45% to 110% in California, Virmani et al. (1982) reported heterosis up to 73% and heterobeltiosis up to 59%, and Sirivastava and Seshu (1982) reported significant heterosis values for the number of panicles per plant and the number of grains per panicle. Kausik and Sharma (1986) observed heterosis values ranging from 27% to 39% in rice yield, attributed to increases in the number of panicles per plant, panicle length, the number of grains per panicle, and grain weight. Gravois et al. (1993) observed heterosis values ranging from -16% to 68% for rice yield. Sürek and Korkut (1996) determined heterosis values ranging from -11% to 42.7%, with an average of 21.1%, and heterobeltiosis values ranging from -12.6% to 33.6%, with an average of 15.2%, for grain yield in plants.

Sürek and Korkut (1996) determined average heterosis values of -1.5% and average heterobeltiosis values of -4.8% for flowering days and average heterosis values of 2.0% and average heterobeltiosis values of -4.5 heterobeltiosis per plant height and for panicle length, they determined average heterosis and heterobeltiosis values ranging from 2.9% to -6.0%. Among the yield components, the highest average heterosis and heterobeltiosis values were found in the number of panicle per plant at 11.9% and 3.9%, followed by the number of grains per panicle at 10.0% and 2.0%, and the weight of 1000 seeds at 1% and -6.4%.

Rukmini et al. (2014) obtained 245.83% heterosis and 197.6% heterobeltiosis for rice yield in plants, while observing high heterosis for 50% flowering days, panicle length, and the grain number per panicle, and low heterosis for number of panicle in plants. Again, Rukmini et al. (2017), in another study, estimated high heterosis values for rice yield, number of filled grains per panicle, plant height, and the number of fertile panicles per plant, and low heterosis values for panicle length. Vanaja and Babu (2004) observed significant heterosis values in rice yield, the grain number per panicle, the panicles number per square meter, and panicle length. Whereas, Babu et al. (2010) similarly observed significant heterosis in rice yield, stem length, the number of filled grains per panicle, and the panicle number per plant.

Joshi (2001) identified high heterobeltiosis values ranging from -5% to 139% for rice yield in Nepal. Parihar and Pathak (2008) observed high heterosis values for rice yield, plant height, thousand grain weight, and the grain number per panicle in India. However, Veerasha et al. (2013) reported heterosis values of -57.26% and 421.22% for rice yield, along with heterobeltiosis values of 59% and 304.4%. Joshi (2003), determined heterobeltiosis ranging from -55% to 139% for rice yield, stating that this was due to the heterotic effect observed in the panicles number per pant, the grain number per panicle and panicle length.

The heritability is an important tool for breeders in selection, helping them determine the source of variation observed in the populations and whether that variation is genetic or environmental. In a broad sense, the degree of heritability reflects variation arising from different gene effects, while in a narrow sense, it specifically refers to genetic variation derived from additive gene effects and can be preserved in pure lines. High narrow sense heritability for flowering days (64%), plant height (75%), panicle length (63%), and 1000 grain weight (86%), moderate heritability for grains number per panicle (56%), and low heritability for panicle number (26%) and rice yield per plant (6%) by Surek and Korkut (1998). Chongkid (1997) determined narrow-sense heritability values of 12.10% for plant height, 6.90% for fertile panicle number, and 23.6% for rice yield per plant. Kou and Liu (1987) determined a narrow-sense heritability (61%) for rice yield. Verica et al. (2003) estimated narrow-sense heritability for the number of fertile panicles per plant (19-30%), the grain number per main panicle (29-55%), and plant height (46-82%). Hefena et al. (2016) estimated narrow-sense heritability for panicle number, the number of fertile grains per panicle, 1000-grain weight, and grain yield in plants at low to moderate levels. Al-Daej (2022) estimated very low heritability estimates in a narrow sense for the maturity days (3.03%), panicle length (3.61%), and 1000-grain weight (3.62%). Viswabharathy et al. (2023) determined a moderately narrow inheritance coefficient of 49.72% for the flowering days, 42.12% for plant height, 53.08% for the number of fertile panicle, and 48.80% for the number of fertile grains per panicle.

The objective of this study is to determine the heterosis and heterobeltiosis values determined for rice yield and yield components in three different hybrid combinations created using four different rice varieties, and to estimate the narrow-sense heritability examined characters. According to obtained result in this study, some recommendations will be done for hybrid rice breeding.

MATERIALS AND METHODS

Material

In this study, Turkish rice varieties Halilbey, Gemici, and Aga developed by the Edirne Trakya Agricultural Research Institute were used in crossbreeding studies, along with the Italian-origin Vasco

variety developed by Leonardo S.R.L. The cross combinations Halilbey x Aga, Halilbey x Gemici and Halilbey x Vasco were created.

The hybrid combinations obtained among the parents in 2018 are presented in Table 1. The reason for creating the Halilbey x Aga hybrid was to combine the characteristics of short stature, large grains, and higher rice yield. On the other hand, the Halilbey x Vasco hybrid was developed with a focus on short stature and high rice yield. Additionally, the Halilbey x Gemici hybrid was developed considering short stature, high yield, and grain quality. The use of the Halilbey rice variety as the main parent in these three hybrid combinations is due to Halilbey's high yield potential. In 2020, observations and measurements were conducted on plants obtained by growing the F₁ generations of these combinations alongside their parents.

Table 1. Hybrid combinations used in the experiment

No	Pedigree No.	Parent	Purpose of crossing
1	2018019-AB59	Halilbey x Aga	Short plant height, strong culm, large grain
2	2018019-AB58	Halilbey x Vasco	Short plant height, high yield
3	2018019-AB57	Halilbey x Gemici	Short plant height, high yield, grain quality

Crossing

In the 2018 rice growing season, the plants were taken off from the field and transplanted in the pot in early morning time. The emasculation process was carried out in the afternoon of the same day, and the hybridization process was carried out around noon time the following day.

Cultivation Techniques

The F₁ hybrids and their parents were grown together during the 2020 rice planting season in the village of Tuzakçı, Gönen district, Balıkesir. The transplanting method was used as the planting method to minimize environmental effect. After 30 days the seedling transferred from seed bed to the field. Transplanting was done 25 x 25 cm planting density with one plant each hill. Fertilization was carried out at a rate of 180 kg of pure nitrogen and 60 kg of pure phosphorus per hectare. One-third of the nitrogen and all of the phosphorus were applied to the soil before transplanting, one-third of the remaining nitrogen was applied 15 days later, and the remaining one-third was applied 35 days later of transplanting. Irrigation and other cultivation techniques were carried out according to traditional rice cultivation practices.

Ten plants were labeled from the parent and the F₁ plants, observations and measurements were made. In these plants, observations and evaluations were made for the flowering and maturation days,

plant height, panicle length, number of panicles per plant, the number of grains per panicle, rice thousand-grain weight, and rice yield per plant.

Heterosis ve Heterobeltiosis

The following formulas were used to estimate heterosis and heterobeltiosis.

$$(H) \text{ Heterosis (\%)} = \frac{F_1 - MP}{MP} * 100 \quad (Hb) \text{ Heterobeltiosis (\%)} = \frac{F_1 - P_B}{P_B} * 100$$

(Fonseca and Patterson, 1968; Hallauer and Miranda, 1981)

F_1 = The average value of a hybrid

MP = Parental average

$F_1 - MP$ = Difference between F_1 and parent average

P_B = Average of parent

Narrow Sense Heritability

The narrow sense heritability of traits studied using parent-offspring regression (Falconer, 1981).

RESULTS AND DISCUSSION

As with all cereals, in hybrid breeding or conventional rice breeding, having information about the yield components and traits contributing to yield, as well as their performance and inheritance patterns, provides significant benefits to breeders. This study was conducted to obtain information about yield components and the heterotic performance and genetic values of certain traits that can be considered in hybrid rice breeding. For this purpose, three hybrid combinations were used.

The average values of the examined traits in the parents and F_1 plants are presented in Table 2. According to the results obtained, the highest heterosis and heterobeltiosis values were observed in the panicle number per plant and rice yield in plant. The lowest heterosis and heterobeltiosis values were determined for the number of grain per panicle and 1000-grain weight (Table 3).

Table 2. The average values of the parents and hybrids for the examined traits.

	Number of days to flowering (days)	Number of days to maturity (days)	Plant Height (cm)	Panicle Length (cm)	Number of panicles per plant	Number of Grains per Panicle	Rice 1000 grains (g)	Rice Yield per Plant (g)
Halilbey	97.1 ± 1.2	131.6 ± 1.6	105.1 ± 2.8	17.5 ±1.7	7.9 ±4.1	108.6 ±10.1	26.1 ±0.6	24.5 ±12.2
Aga	91.8 ±3.8	139.0 ±1.5	88.5 ±2.9	19.2 ±1.9	6.0 ±1.89	79.1 ±13.1	37.5 ±0.7	15.2 ±5.6
Gemici	96.4 ±3.4	132.3 ±3.0	102.8 ±4.1	18.1 ±2.7	12.3 ±3.5	110.0 ±21.4	32.5 ±0.4	44.2 ±15.8
Vasco	94.6 ±1.0	139.3 ±1.8	79.7 ±3.0	18.2 ±1.5	8.0 ±1.2	89.9 ±10.0	32.5 ±0.9	23.5 ±4.6
HalilbeyXAga	90.3 ±1.6	132.4 ±1.7	107.5 ±3.3	19.9 ±0.9	21.8 ±5.2	111.1 ±13.6	30.3 ±0.5	66.6 ±16.6
HalilbeyXGemici	92.9 ±1.6	139.0 ±3.3	105.2 ±4.2	17.8 ±2.3	21.5 ±2.1	94.0 ±9.0	31.1 ±0.9	63.6 ±8.5
HalilbeyXVasco	91.7 ±0.9	127.0 ±1.4	90.8 ±1.7	20.2 ±1.4	25.8 ±5.4	85.6 ±10.1	30.9 ±0.4	69.6 ±13.9

The highest narrow-sense heritability estimates were obtained for panicle number, rice yield, and number of days to maturity. Medium narrow-sense heritability estimates were obtained for plant height and 1000-grain weight, while low narrow-sense heritability estimates were obtained for flowering days, panicle length, and the grains number per panicle.

In hybrid combinations, average heterosis values of -4.23% (-4.39% and -3.98%) and average heterobeltiosis values of -5.63% (-4.33% and -7.00%) were observed for the number of flowering days. Srek and Korkut (1996) and Rukmini et al. (2014) obtained similar results. A negative heterobeltiosis value for the number of flowering days indicates that the hybrid offspring produce inflorescences earlier than the parents. In the HalilbeyxAga and HalilbeyxVasco combinations, values indicating earlier maturity were also obtained for the number of days to maturity, similar to those for the number of days to flowering. However, in the HalilbeyxGemici combination, positive heterosis and heterobeltiosis were detected for maturity days.

For plant height, an average heterosis of 3.49% between -1.73% and 11.05% and an average heterobeltiosis of -3.74% between -13.61% and 0.10% were determined. However, heterosis and heterobeltiosis values were negative in the HalilbeyxVasco hybrid. This situation is attributed to the genetically reflected short stature of the Vasco variety in the F₁ hybrid. Srek and Korkut (1996) also reported similar heterosis and heterobeltiosis values in their studies. On the other hand, Rukmini et al. (2014) determined low heterosis values for plant height, while Joshi (2001) reported high heterosis values. The negative heterobeltiosis in plant height indicates that F₁ hybrids will be shorter than their parents.

For plant length, an average heterosis of 7.21% was determined between 0.00% and 13.17% and 10.99%, with an average heterobeltiosis of 4.33%. In contrast, heterosis and heterobeltiosis values for the HalilbeyxGemici hybrid were determined as 0.00% and -1.66%, respectively. This may be due to the fact that the Halilbey and Gemici varieties have similar plant length values. This situation did not result in significant changes in the F₁ hybrid. Sürek and Korkut (1996) observed average heterosis values of 2.90% and heterobeltiosis values of -6.00% for plant length. On the other hand, Rukmini et al. (2014) detected low heterosis for plant length. A positive heterosis value for plant length indicates longer plant values in hybrids, which is a desirable trait for high rice yields.

Table 3. Heterosis (%), heterobeltiosis (%) and narrow sense heritability values determined for the examined characters

Hybrid Combinations	Number of days to flowering			Number of days to maturity			Plant Height			Panicle length		
	He(%)	Hb(%)	H ²	He(%)	Hb(%)	H ²	He(%)	Hb(%)	H ²	He(%)	Hb(%)	H ²
HalilbeyxAga	-4,39	-7,00	0,16	-2,14	-4,75	1,30	11,05	2,28	1,29	8,45	3,65	0,22
HalilbeyxGemici	-3,98	-4,33	0,21	5,34	5,06	0,63	1,15	0,10	0,03	0,00	-1,66	0,36
HalilbeyxVasco	-4,33	-5,56	0,39	-6,24	-8,83	0,32	-1,73	-13,61	0,30	13,17	10,99	0,20
Avarage	-4,23	-5,63	0,26	-1,01	-2,84	0,75	3,49	-3,74	0,54	7,21	4,33	0,26
	Number of panicles per plant			Number of grains per panicle			Thousand Rice Grains Weight			Rice Yield per Plant		
	He(%)	Hb(%)	H ²	He(%)	Hb(%)	H ²	He(%)	Hb(%)	H ²	He(%)	Hb(%)	H ²
HalilbeyxAga	213,67	175,95	0,61	18,38	2,30	0,37	-4,72	-19,20	0,63	235,52	171,84	0,94
HalilbeyxGemici	112,87	74,8	0,36	-13,17	-13,73	0,07	6,14	-4,31	0,93	85,15	43,89	0,48
HalilbeyxVasco	224,53	222,5	1,59	-13,75	-21,18	0,30	5,46	-4,92	0,05	190,00	184,00	1,07
Avarage	183,69	157,75	0,9	-2,85	-10,87	0,2	2,29	-9,48	0,54	170,22	133,24	0,83

An average heterosis of 183.69% and heterobeltiosis of 157.75% were determined for the number of panicle in plants, ranging from 112.87% to 224.53%. However, heterosis and heterobeltiosis values were lower in the HalilbeyxGemici combination compared to other combinations. This situation may be attributed to the high tillering potential of the Gemici variety. Similarly, Sirivastava and Seshu (1982) and Rukmini et al. (2017) determined similar heterosis values for the number of panicle per plants. Similarly, Sürek and Korkut (1996) reported high heterosis and heterobeltiosis for this trait. Whereas, Rukmini et al. (2014) observed low heterosis for this trait.

For the number grains per panicle, heterosis values ranged from -13.17% to 18.38%, with an average of -2.85%, and heterobeltiosis values ranged from -21.18% to 2.30%, with an average of -10.87%. The heterosis and heterobeltiosis values for the HalilbeyxAga hybrid are positive. Additionally, high heterosis and heterobeltiosis values in a positive direction were determined for the panicle number per the plant in this combination. This suggests that this combination is not significantly affected by environmental factors in terms of these two yield components. Therefore, it can be considered as a combination suitable for hybrid breeding. For this trait, Sürek and Korkut (1996) obtained low heterosis and heterobeltiosis values, while Rukmin et al. (2014) and Parihar and Pathak (2008) determined high heterosis values. The high heterotic effect in the number of grains per panicle will positively produce contribution to rice yield.

For the thousand-grain weight of rice, heterosis values ranged from -4.72% to 6.14%, with an average of 2.29%, and heterobeltiosis values ranged from -19.20% to -4.31%, with an average of -9.48%. Both heterosis and heterobeltiosis values were negative for the HalilbeyxAga hybrid. This is due to the large grain size of the Aga variety, one of the varieties in the combination. On the other hand, in the other two combinations, heterosis was positive and heterobeltiosis was negative. This is thought to be due to the grain weights of the varieties in the combination being at the same level. Sürek and Korkut (1996) obtained similar results for this character. However, Parihar and Pathak (2008) observed high heterosis for thousand grain weight.

For rice yield, heterosis values ranging from 85.15% to 190.0%, with an average of 170.22%, and heterobeltiosis values ranging from 43.89% to 184.00%, with an average of 133.24%, were determined. The heterosis and heterobeltiosis values obtained for rice yield were positive in all three combinations. However, the values detected in the HalilbeyxGemici combination were at lower levels. This may be due to the high yield potential of the Gemici variety. Similarly, for rice yield, Virmani et al. (1982) reported 73% heterosis and 59% heterobeltiosis, Kausik and Sharma (1986) reported heterosis values between 27% and 30%, Gravois et al. (1993) reported heterosis values between -16% and 68%, Sürek and Korkut (1996) reported an average of 21.1% heterosis and 15.2% heterobeltiosis, and Veerasha et al. (2013) found heterosis values ranging from -57.26% to 421.22% and heterobeltiosis values ranging from 59% to 304.4%. Additionally, Joshi (2003) observed heterobeltiosis ranging from -5% to 139%.

In this study, the increase in heterosis and heterobeltiosis in rice yield was attributed to the increase in heterosis observed in the number of panicles for plant, with an additional contribution from the increase in panicle length. Similar results were reported by Kausik and Sharma (1986), Vanaja and Babu (2004), and Joshi (2003). The heterosis and heterobeltiosis determined for the filled grains per panicle were negative, and their contribution to rice yield increase was also negative. However, Kausik and Sharma (1986), Venaja and Babu (2004), and Joshi (2003) obtained opposite results for this trait.

The number of maturity days resulted in an average narrow-sense heritability value of 75%, followed by the number of flowering days (26%), the number of days to maturity was considered more suitable for selection. However, Sürek and Korkut (1998) determined high heritability for the number of flowering days, while Viswabharathy et al. (2023) found moderate heritability. Al-Daej (2022), obtained low heritability for maturity days.

The average narrow-sense heritability for plant height is 54%, while that for panicle length is 26%. Plant height has a safer genetic structure the panicle length. Sürek and Korkut (1998) and Verica et al. (2003) estimated high heritability for these two traits, while Chongkid (1997) estimated low heritability for plant height and Al-Daej (2022) for panicle length.

Among examined characters, the highest narrow-sense heritability was estimated in the panicle number per plant, averaging 90%, it followed by rice 1000-grain weight (54%) and the number of filled grains per panicle (20%). On the contrary, Sürek and Korkut (1998) 26%, Chongkid (1997) 12.10%, Verica et al. (2003) 19.30%, and Viswaloharathy et al. (2023) 53.08%, a narrow sense heritability reported for the fertile panicles per plant respectively while for the grain number per panicle, narrow sense heritability values by Sürek and Korkut (1998) 56%, Verica et al. (2003) 29–55%, and Viswaloharathy et al. (2023) 49.72%. For thousand-grain weight, Sürek and Korkut (1998) determined a high heritability of 86%, while Verica et al. (2003) and Al-Daej (2022) obtained results similar to those of this study.

The average narrow sense heritability value for rice yield was estimated 83%. Kou and Liu (1987) found similar result for rice grain yield. However, low narrow sense heritability for rice yield was recorded by Sürek and Korkut (1998), Chongkid (1987), and Hefena et al. (2016).

CONCLUSION

According to the results of this study, among the yield components, the number of panicle per plant had the highest heterosis and narrow sense heritability values, therefore, this trait may be used as a selection criteria in heterosis breeding in rice. At the same time, flowering day and panicle length should be taken into account in the selection.

Additional Declaration

Conrobition of authors

1.Authors: His contribution is %60. He conducted all experiment works statistic analyzing, collection literature and writing the paper etc.

2.Authors: His controbaton is %20. He planned the experiment design in the field. He did recommendations to the first author for field observation and data collection. He reviewed draft paper and he did necessary collection in the paper.

3.Authors: Her contribution is %20. She planned and designed the experiment. She made recommendations for observation data analyzing. She supervised for all works from beginning of the study to finish. She also did final review in the paper.

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Artificial Intelligence Statement

No artificial intelligence support was used in any part of this study.

Conflict Of Interest Statement

The authors declare that they have no conflict of interest regarding the publication of this study.

Ethical Statement

This study was conducted in accordance with the principles of Pen Academic Publishing Research Ethics Policy throughout all stages of the process.

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