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ABOUT

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Assessing Ergonomic Risks in Worker Postures: The Case of Belt Conveyor Assembly

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ABSTRACT

In the study, the working conditions that challenged the employees during the assembly process in a factory where a belt conveyor was manufactured were analyzed using the REBA method. As a result of the evaluations, these challenging working postures were examined in seven stages. It was observed that the employees performed the assembly process primarily in squatting and heavily leaning postures. While the REBA score was recorded as 2 in the six stages related to assembling the carrier rollers onto the chassis of the conveyor belt, the highest REBA score, 13, was found in the seventh stage, which involved assembling the electric motor. It was recommended that the assembly operations be conducted on a movable platform with adjustable length and height, and that an additional platform capable of horizontal movement be used during the assembly of the electric motor. This approach would improve the risk protect for workers and help safeguard their health.

Keywords: *Belt conveyor assembly, REBA method, Worker posture evaluation, Ergonomic risk analysis*

INTRODUCTION

Professionals working in the field of ergonomics investigate topics such as risks involved in work activities, posture-related load, the effects of vibration, tool usage, connections, improper postures, the frequency and duration of movements, work irregularities, and the design of ergonomic workstations (Joshi and Deshpande, 2019).

Work-Related Musculoskeletal Disorders are prevalent in industrial settings, particularly where employees are engaged in physically demanding tasks. The occurrence and nature of these disorders vary significantly across different sectors (Storheim and Zwart, 2014).

Musculoskeletal disorders are among the most prevalent occupational challenges in both developed and developing nations, significantly impacting the industrial and service sectors. They contribute to higher healthcare expenses, increased wage compensation, reduced productivity, and a lower quality of life. Annually, these disorders affect billions globally. Work-related musculoskeletal disorders (WMSDs) arise from the interaction of multiple risk factors and can be classified into individual, psychosocial, and physical categories (Al Madani and Dababneh, 2016). The primary cause of WMSDs is repetitive stress that accumulates over time (Joshi and Deshpande, 2020). The global prevalence of musculoskeletal disorders (MSD) is reported to range from 14% to 42% (Sharma, 2012). Industries, particularly in developing countries with abundant and low-cost labor, heavily depend on human labor. However, this focus on cost reduction often leads to the neglect of ergonomic conditions. This underscores the importance of raising awareness about ergonomic risks, identifying their root causes, and implementing effective preventive measures. Without such precautions, employees are exposed to varying degrees of risk-ranging from low to very high-depending on the intensity and nature of their work. Ergonomic interventions are essential to minimize workers' exposure to high-risk factors, thereby reducing the prevalence of occupational diseases and workplace health and safety concerns. Ultimately, these efforts aim to optimize workers' health, safety, and productivity while improving their comfort, aligning with the core objective of ergonomics (Niu, 2010).

The REBA (Rapid Entire Body Assessment) method is one of the most widely recognized and commonly utilized observational ergonomic assessment tools across diverse industries and service sectors. Among all ergonomic assessment methods, REBA is highly generalized and broadly applied in many sectors (Hita-Gutiérrez *et al.*, 2020). It is a practical tool designed to evaluate the entire body, providing a numerical representation of the risk associated with specific working postures or movements (Çoker and Selim, 2019).

In our country, studies have been conducted on the posture analysis of workers in various sectors using the REBA method. It has been applied in replication tasks at the Trabzon-of forest nursery in the agricultural sector (Ünver-Okan and Kaya, 2015) and in corn production (Geniş and Sümer, 2021), in the construction sector (Obuz, 2016), in cable manufacturing factories (Ulutaş and Gündüz, 2017), in expansion tank manufacturing in the metal industry (Özoğul *et al.*, 2018), on combi boiler assembly lines (Gürleyen and Kahya, 2018), in bolt manufacturing factories (Sever and Deste, 2021), in textile enterprises (Çoker and Selim, 2019) and (Akyol, 2022), in the production line of the heavy metal industry (Tarakçı *et al.*, 2020), in casting workshops (Erdemir and Eldem, 2020), in the food sector (Kılıç and Çetin, 2021)and (Baş and Yapıcı, 2020), in the automotive sub-industry (Cakmak and Esen, 2023), in the assembly process of hay rakes (Gönen *et al.*, 2017a), in transformer operations (Gönen *et al.*, 2017b), in elevator production (Oral *et al.*, 2018) and in bolt factories (Sever and Deste, 2021).

This study aims to improve the unsuitable postures observed during the assembly process of a conveyor belt, which is part of the product design of a micro-scale enterprise. No similar study has been encountered in the literature on this topic. Analyses were conducted to determine the strain and muscle activations experienced by workers during the assembly process. The workers' risk levels for Musculoskeletal Disorders (MSDs) were assessed using the REBA method.

MATERIALS and METHODS

An Ethics Committee Approval Certificate was obtained with the decision dated 28.06.2024 and numbered E.781681 from the Scientific Ethics Review Board of Selçuk University Faculty of Agriculture.

The research was conducted in 2024 at KDM Makina, a company located in Konya, Turkey. The firm manufactures belt conveyors in various sizes. This is a micro-scale enterprise with a total of three workers, all of whom are involved in the assembly process.

The belt conveyor examined in this study was manufactured for a foundry, where it transports sand from the bunkers to the molding machine. The conveyor is 3 000 mm in length and 1 080 mm in width, with a belt width of 800 mm. A schematic view of the belt is provided in Figure 1. During the production phase, all connection holes in the chassis were cut using a laser, and the chassis was bent using a press brake. The assembly consists solely of bolt-nut connections.

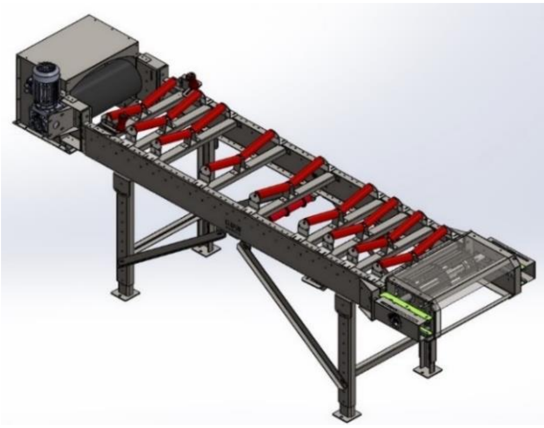


Figure 1*. Schematic view of the belt chassis.*

Workers are exposed to Musculoskeletal Disorders (MSDs) during the assembly process. In the study, ergonomic analyses were conducted for the workers' postures during the production process of the conveyor belt, and their postures were evaluated using the REBA method. The assembly process was examined under seven sections. These stages are:

1. Initial assembly of the main chassis,
2. Connection of the roller side lugs to the main chassis,
3. Secondary assembly of the main chassis,
4. Assembly of the roller middle lugs
5. Mounting of drums,
6. Installation of the carrier rollers
7. Assembly of the electric motor

After these assembly operations, the installation of the belt conveyor and the belt are installed. In this study, ergonomic risks were evaluated using the REBA method, with all tables provided by Hignett and McAtamney (2000). The flowchart of the schematized REBA method is shown in Figure 2. According to this method, body parts are divided into two groups, A and B, when determining the REBA score.

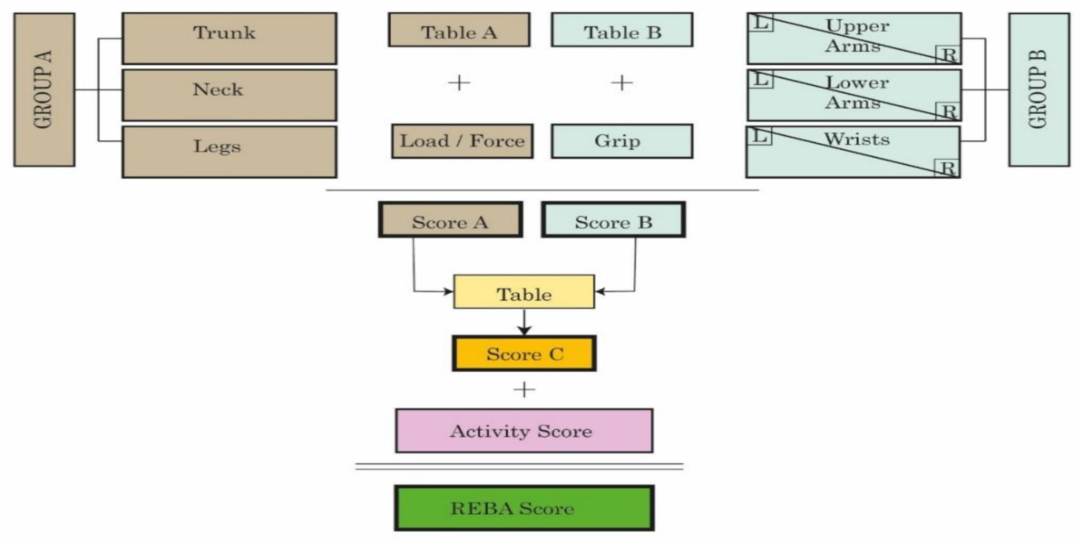
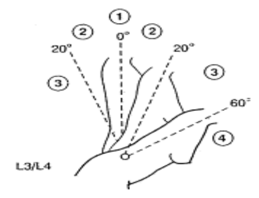
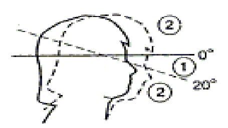


Figure 2. Flowchart of the REBA method.

Group A consists of limbs, trunk, neck and legs. Separate scores are determined for the trunk, neck and legs according to the worker's posture position (Figure 3). Using Table A, these individual scores, combined with the posture angles, are converted into a numerical format (Table 1).

Trunk			
Movement	Score	Change score	
Upright	1	+1 if twisting or side flexed	
0^0-20^0 flexion $>0^0$-20^0 extension	2		
20^0-60^0 flexion $>20^0$ extension	3		
$>60^0$ flexion	4		

Neck			
Movement	Score	Change score	
0^0-20^0 flexion	1	+1 if twisting or side flexed	
$>20^0$ flexion or extension	2		

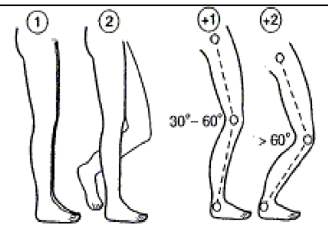
Legs			
Position	Score	Change score	
Bilateral weight bearing, walking or sitting	1	+1 if knee(s) between 30^0 and 60^0 flexion	
Unilateral weight bearing Feather weight bearing or an unstable posture	2	+2 if knee(s) are $>60^0$ flexion (n.b. Not for sitting)	

Figure 3. Diagram of group A body parts and corresponding scores.

After obtaining the numerical scores for Group A, the A score is calculated by adding the Load/Force score provided in Table 2 (Table 1).

Table 1*. REBA method: Group A.*

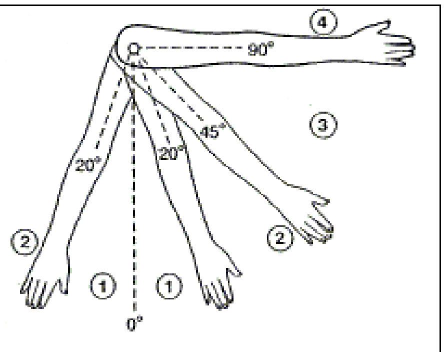
Table A	Neck												
		1				2				3			
	Legs												
		1	2	3	4	1	2	3	4	1	2	3	4
Trınk Poisture Score	1	1	2	3	4	1	2	3	4	3	3	5	6
	2	2	3	4	5	3	4	5	6	4	5	6	7
	3	2	4	5	6	4	5	6	7	5	6	7	8
	4	3	5	6	7	5	6	7	8	6	7	8	9
	5	4	6	7	8	6	7	8	9	7	8	9	9

Table 2*. Load / Force score.*

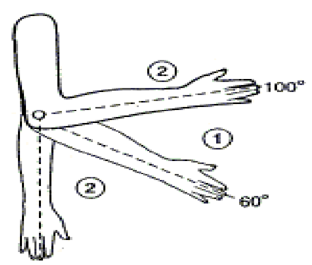
0	1	2	+1
<5 kg	5-10 kg	>10 kg	Sock or rapid build uo of force

Group B consists of upper arms, lower arms and wrists. According to the REBA method, separate scores are determined for the upper arm, lower arm, and wrist for both the right and left limbs simultaneously, based on the worker's posture, as shown in Figure 4.

Upper arms Movement	Score	Change score
20^0 extension to 20^0 flexion	1	+1 arm is: abducted, rotated +1 if shoulder is raised -1 if leaning supporting weight of arm or if posture is gravity assisted
$>20^0$ extension 0^0-20^0 flexion	2	
45^0-90^0 flexion	3	
$>90^0$ flexion	4	



Lower arms Movement	Score
60^0-100^0 flexion	1
$<60^0$ flexion or $>100^0$ flexion	2



Wrists Movement	Score	
0^0-15^0 flexion/extension	1	+1 if the wrist are on either radial or ulnar deviation
$>15^0$ flexion/extension	2	

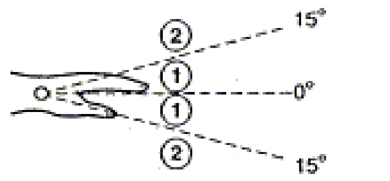


Figure 4*. Diagram of group B body parts and corresponding scores.*

The first step in determining the Group B score is to combine the arm and wrist angle score values from the table (Table 3). To calculate the final B score, the grip score value provided in Table 4 is added to the Group B score.

Table 3. *REBA method: Group B table.*

Table B	Lower arm					
		1			2	
	Wrist					
		1	2	3	1	2	3
Upper Arm Score	1	1	2	2	1	2	3
	2	1	2	3	2	3	4
	3	3	4	5	4	5	5
	4	4	5	5	5	6	7
	5	6	7	8	7	8	8
	6	7	8	8	8	9	9

Table 4. *Grip score.*

0 Good	1 Fair	2 Poor	3 Unacceptable
Well-fitting handle and a mid-rage, power grip	Hand hold acceptable but not ideal or coupling is acceptable via another part of body	Hand hold not acceptable although possible	Awkward unsafe grip, no handles Coupling is un acceptable using parts of the bady

The A and B scores obtained from the REBA flow chart (Figure 1) are combined using the C Table, as shown in Table 5. This process yields the final C score in the method.

Table 5. *REBA method C table.*

Score A	Table C Score B											
	1	2	3	4	5	6	7	8	9	10	11	12
1	1	1	1	2	3	3	4	5	6	7	7	7
2	1	2	2	3	4	4	5	6	6	7	7	8
3	2	3	3	3	4	5	6	7	7	8	8	8
4	3	4	4	4	5	6	7	8	8	9	9	9
5	4	4	4	5	6	7	8	8	9	9	9	9
6	6	6	6	7	8	8	9	9	10	10	10	10
7	7	7	7	8	9	9	9	10	10	11	11	11
8	8	8	8	9	10	10	10	10	10	11	11	11
9	9	9	9	10	10	10	11	11	11	12	12	12
10	10	10	10	11	11	11	11	12	12	12	12	12
11	11	11	11	11	12	12	12	12	12	12	12	12
12	12	12	12	12	12	12	12	12	12	12	12	12

There is a lack of information regarding whether the task is performed in a moving or stationary position. This gap is addressed by adding the activity score of the worker to the C score obtained (Table 6).

Table 6. *Activity score.*

+1	1 or more body parts are static, e.g. held for longer than 1 min
+1	Repeated small range actions, e.g. repeated more than 4 times per minute (not including walking)
+1	Action causes rapid large range changes in postures or an unstable base

A REBA score is obtained by adding the activity score to the C score. The ergonomic risk assessment of the working conditions is then performed using the resulting REBA score. This score is evaluated according to the REBA risk rating table, shown in Table 7. The REBA risk rating consists of 5 levels, ranging from 0 to 4, with scores separated by numerical values from 1 to 15. The ergonomic risk assessment of the working conditions is determined based on the obtained REBA score.

Table 7. *REBA score table.*

Action level	REBA score	Risk level	Action(including further assessment)
0	1	Negligible	None necessary
1	2–3	Low	May be necessary
2	4–7	Medium	Necessary
3	8–10	High	Necessary soon
4	11–15	Very high	Necessary now

RESULTS and DISCUSSION

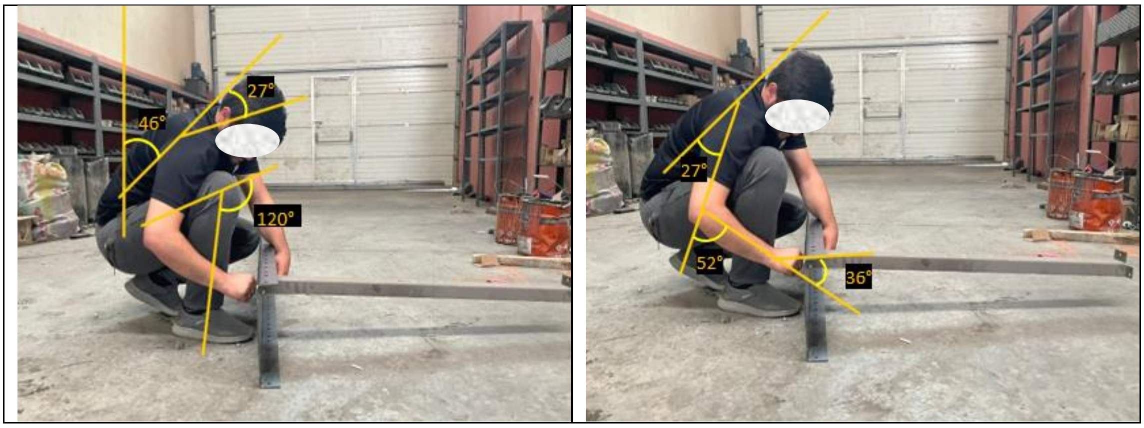
The ages, weights, and heights of the three workers employed in the micro-scale enterprise were determined as 37, 32, and 24 years; 65, 85, and 80 kg; and 171, 178, and 172 cm, respectively. Additionally, their work experience is 2, 2, and 3 years, and their educational background includes one primary school graduate and two high school graduates.

During the assembly of the belt conveyor, REBA analysis was conducted based on the process steps, assessing the body, neck, leg, upper and lower arm, and wrist postures. The REBA analysis results obtained during the initial connection of the main chassis are shown in Table 8.

At the start of the main chassis assembly, the worker works in a squatting position, placing the greatest strain on the body, as indicated in Table A. In this study, the REBA score was determined to be 5, indicating a medium risk level. Precautionary measures are necessary for this work posture, which is maintained throughout the day.

Table 8. *Initial assembly of the main chassis.*

<table>
<tr><td colspan="2">Group A</td><td>Table A</td><td colspan="2">Group B</td><td>Table B</td></tr>
<tr><td>Body</td><td>3</td><td rowspan="4">5</td><td>Upper arm</td><td>2</td><td rowspan="4">3</td></tr>
<tr><td>Neck</td><td>2</td><td>Lower arm</td><td>2</td></tr>
<tr><td>Legs</td><td>2</td><td>Wrist</td><td>2</td></tr>
<tr><td>Load/Force</td><td>-</td><td>Clutch</td><td>-</td></tr>
<tr><td>A score</td><td colspan="2">5</td><td>Score B</td><td colspan="2">3</td></tr>
<tr><td colspan="3">C score</td><td colspan="3">4</td></tr>
<tr><td colspan="3">Activity score</td><td colspan="3">1</td></tr>
<tr><td colspan="3">REBA score</td><td colspan="3">5</td></tr>
<tr><td colspan="3">Degree</td><td colspan="3">2</td></tr>
</table>



The results obtained from connecting the roller side lugs to the main chassis are shown in Table 9. In this position, the work is performed on the floor with the worker standing and bent over, and the task is repeated throughout the day. In this process, the greatest strain occurs in the trunk and upper arms. By adding the activity score (2) to the obtained C score (5), the REBA score was determined to be 7. The risk level for this working posture is medium, and precautions are required to improve this posture. Oral *et al.* (2018) reported that marking the belt holes of a bucket elevator was performed on the ground, with a REBA score of 10 determined for this task. Due to the high frequency of repetitive movements during this assembly process, it is necessary for workers to rotate tasks. This approach would help reduce the repetitive movements performed by the workers.

As observed in Table 8 and Table 9, workers perform these assembly processes on the ground. Strains are evident in their trunks, neck, and legs. Özoğlu *et al.* (2017) determined a REBA score of 8 for the task of packaging metal washers and reported that the lack of an appropriately elevated press bench caused strain in the lower back, upper back, and arms. Gönen *et al.* (2017b) found that during transformer assembly, workers experienced significant strain in the trunk, neck, legs, and upper arms due to squatting and bending postures, with a REBA score of 12. İn another research, Erdemir and Eldem (2020) conducted an ergonomic analysis of work postures during the ladle preparation stage in a foundry using the REBA method and identified a REBA score of 10.

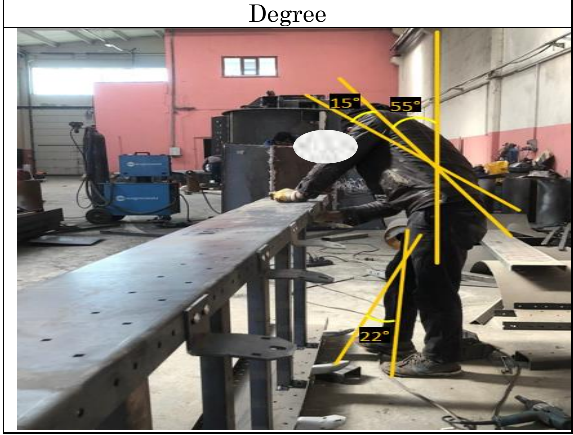
***Table 9**. Connection of the roller side lugs to the main chassis.*

Group A		Table A	Group B		Table B
Body	4	5	Upper arm	3	4
Neck	1		Lower arm	2	
Legs	2		Wrist	1	
Load/Force	-		Clutch	-	
A score	5		Score B	4	
C score			5		
Activity score			2		
REBA score			7		
Degree			2		

In the third stage, the connection of the main chassis is completed. The A (5) and B (2) scores for the two main chassis, joined by bolts and nuts, were matched in the C table, resulting in a score of 4 (Table 10). With the addition of the activity score, the REBA score was determined to be 5. The risk level for this task falls within the range of 4-7, indicating a medium risk level. As per the REBA guidelines, precautions should be taken to mitigate the risks associated with this posture.

***Table 10**. Second assembly of the main chassis.*

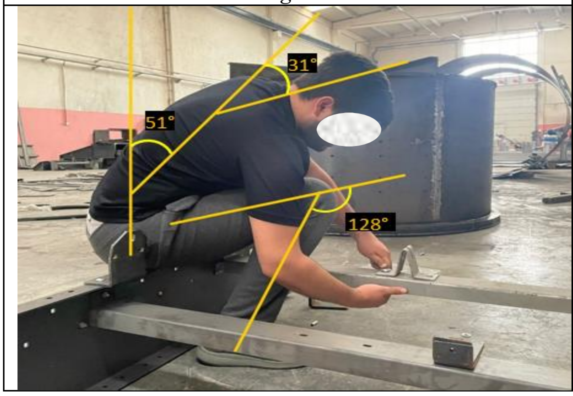
Group A		Table A	Group B		Table B
Body	4	5	Upper arm	2	2
Neck	1		Lower arm	1	
Legs	2		Wrist	2	
Load/Force	-		Clutch	-	
A score		5	Score B		2
C score			4		
Activity score			1		
REBA score			5		
Degree			2		



The REBA analysis of the roller middle lug connection in the fourth stage is shown in Table 11. It is observed that the trunk, legs, upper arms, and wrists experience significant strain during this assembly. After adding the activity score to the C score, the REBA score was determined to be 8. This score indicates a high-risk level, and precautions should be taken promptly.

***Table 11**. Reel middle lug assembly.*

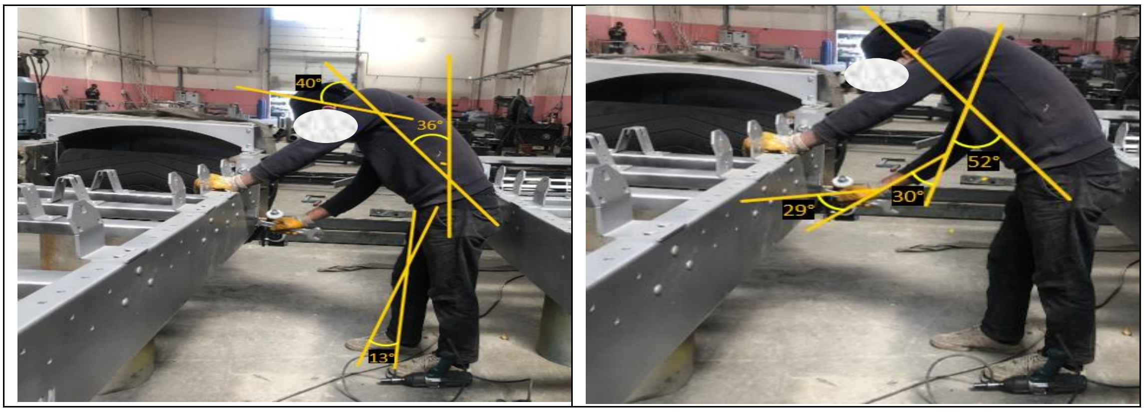
Group A		Table A	Group B		Table B
Body	3	6	Upper arm	3	4
Neck	2		Lower arm	1	
Legs	3		Wrist	2	
Load/Force	-		Clutch	-	
A score	6		Score B	4	
C score			7		
Activity score			1		
REBA score			8		
Degree			3		



The REBA score table determined in the fifth assembly stage, which involves connecting the drums to the chassis, is shown in Table 12. Upon examining the relevant table, the Table A score was found to be 5 by evaluating the leg, neck, and trunk posture positions. The B score was determined by assessing the lower arm, wrist, and upper arm posture positions, resulting in a score of 5. Based on these scores, the C score was calculated to be 6. After adding the activity score, the REBA score was determined to be 8. Considering the risk level range, this score falls within the high-risk category (8-10). According to the REBA guide, precautions should be taken promptly. Aksüt *et al.* (2020) reported that hand-operated workers in the industrial sector are exposed to critical physical strain that leads to musculoskeletal disorders, with lifting, poor posture, and repetitive movements identified as the primary causes of these disorders. Therefore, it is necessary to improve the posture during the drum connection process.

***Table 12**. Drum connection.*

Group A		Table A	Group B		Table B
Body	3	5	Upper arm	3	5
Neck	2		Lower arm	2	
Legs	2		Wrist	2	
Load/Force	-		Clutch	-	
A score	5		Score B	5	
C score			6		
Activity score			2		
REBA score			8		
Degree			3		



The worker stands while assembling the conveyor belt on the chassis of the carrier rollers (Table 13). The A, B, and C scores were found to be 4, 1, and 2, respectively, resulting in a REBA score of 2. The risk level for this working position was determined to be low, with a risk degree of 1. While the risk is low, precautions may still be required. Ayan (2015) determined a REBA score of 2 for flywheel assembly in the automotive sector. The aforementioned assembly stages should be performed on a platform.

Table 13. *Mounting of carrier rollers.*

Group A		Table A	Group B		Table B
Body	2	4	Upper arm	1	1
Neck	2		Lower arm	1	
Legs	2		Wrist	1	
Load/Force	-		Clutch	-	
A score	4		Score B	1	
C score			2		
Activity score			-		
REBA score			2		
Degree			1		

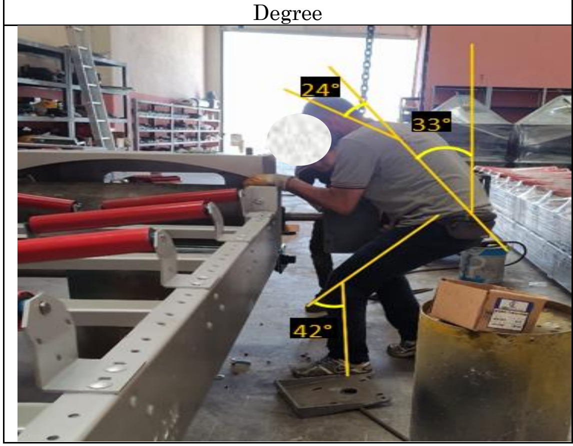
In the final stage of the assembly, the electric motor is mounted (Table 14). Upon examining Table 14, the A score was found to be 8, and the B score was 7. When the A and B scores were matched in the C table, the score was determined to be 10. With the addition of the activity score, the REBA score for the electric motor assembly was calculated as 13. This score corresponds to a degree of 4, indicating a very high-risk level, and according to the REBA guidelines, immediate action must be taken.

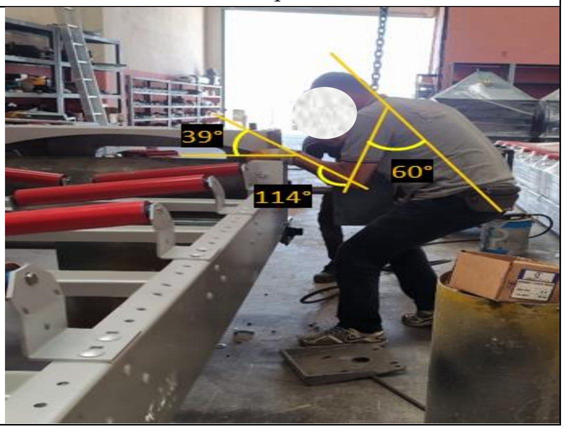
In the final stage of assembly, the electric motor is installed (Table 14). Upon examining Table 14, the A score was determined to be 8 and the B score was 7. When the A and B scores were matched in Table C, the score was determined to be 10. With the addition of the activity score, the REBA score for the electric motor assembly was calculated to be 13.

This score corresponds to a degree of 4, indicating a very high-risk level, and according to the REBA guidelines, immediate action must be taken.
At this stage, the assembly of the electric motor should be carried out on a platform equipped with a slide system capable of horizontal movement.

Table 14. *Mounting of electric motor.*

Group A		Table A	Group B		Table B
Body	3	5	Upper arm	3	5
Neck	2		Lower arm	2	
Legs	2		Wrist	2	
Load/Force	3		Clutch	2	
A score	8		Score B	7	
C score			10		
Activity score			1		
REBA score			13		
Degree			4		





During the assembly of the drum, roller, and electric motor, the belt chassis is placed on a support from both sides. There is a high risk of workplace accidents if any falling incident occurs during the operation.

CONCLUSION

In the study, the production process of a belt conveyor was examined in seven defined stages. The REBA method was used to analyze potential strain on the workers. Excessive loads during assembly were found to cause Musculoskeletal Disorders (MSDs). The lowest REBA score, 2, was recorded during the assembly of the carrier rollers onto the conveyor belt chassis, while the highest REBA score, 13, was recorded during the assembly of the electric motor. The risk level for roller installation was identified as low, whereas the risk level for electric motor installation was determined as very high. Medium and high-risk levels were identified in the working conditions of the other assembly stages. Considering the working conditions, it was concluded that assembly operations should not be performed on the ground but rather on platforms with adjustable length and height. Furthermore, although lifting tasks during the assembly of the drum and electric motor were performed using a crane, it is recommended that the electric motor assembly be carried out on a secondary platform equipped with a slide system capable of horizontal movement. The use of such platforms would reduce risks, prevent unnecessary muscle movements, protect workers' health, shorten production time, and result in cost savings.

Additionally, it is evident that ergonomic training sessions need to be provided within the scope of occupational health and safety (OHS). Thus, workers should be informed about the necessity of correcting their leg postures and avoiding wrist twisting.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

The author declared that the following contributions is correct.

Haydar HACISEFEROĞULLARI: Investigation, methodology, conceptualization, formal analysis, validation, writing-orginal draft, review and editing
Hasan ŞAŞKIN: Data curation, formal analysis, validation, writing, visualization

ETHICS COMMITTEE DECISION

An Ethics Committee Approval Certificate was obtained with the decision dated 28.06.2024 and numbered E.781681 from the Scientific Ethics Review Board of Selçuk University Faculty of Agriculture.

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Modification and Evaluation of Motorized Enset Corm Grinding Machine

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ABSTRACT

This study was executed to redesign and evaluate a motorized enset corm grinding machine. The collected data for tests were analyzed utilizing the Statistix 8 software. The result of the design calculation indicated that the volume of the feeding hopper, the volume of a grinding unit, the length of the belt, the speed ratio, lap angle, shaft diameter, belt tension, torque, the power needed to grind, as well as the force required to grind were obtained as 0.1114 m^3, 0.0796 m^3, 1.18 m, 1.3, 2.95 rad, 40 mm, 1958.6 N, 21.41 Nm, 7 hp, and 298.7 N, correspondingly. The results of the ANOVA tests revealed that the efficiency and percentage loss of the machine were affected by operating speed and feed rate, except for the combined effects, which were due to both factors. The findings revealed that the highest grinding capacity of 894.8 kg h^{-1} was obtained at 2200 rpm operating speed as well as 10 kg min^{-1} feed rate while the lowest grinding capacity of 785 kg h^{-1} was obtained at 2000 rpm operating speed and 15 kg min^{-1} feed rate. The test's results revealed that the maximum grinding efficiency was obtained as 97.9% at 2200 rpm operating speed and 15 kg min^{-1} feed rate, and the minimum grinding efficiency was obtained as 94.3% at 2000 rpm and 10 kg min^{-1} feed rate. The test's result implied that the minimum loss percentage was noted as 4.1% on the operating speed at 2200 rpm and feed rate at 15 kg min^{-1} when operating at 2000 rpm and feed rate at 10 kg min^{-1}, the maximum loss was noted as 7.7%. This redesigned machine was cost-effective because it was fabricated from local source materials. The test results suggested that this redesigned machine was recommendable for growers of enset for grinding the enset corm.

Keywords: *Enset corm, Grinder, Grinding capacity, Efficiency, Percentage loss.*

INTRODUCTION

Enset (*Ensete ventricosum*) is one of the most extensively utilized food in southern Ethiopia and has frequently offered Ethiopians their primary source of food security because of its importance and versatility. It is the primary food source in Ethiopia's highly populated South and Southwestern areas. In the southern region of Ethiopia, it offers a sustainable food supply to roughly twenty-four million people (Blomme *et al.*, 2023). Enset plays a significant role in ensuring access to nutritious food, throughout the year generating income, protecting assets, and maintaining the availability of food. Due to its drought resistance, its adaptable plant can be grown to reduce risk and provide food for humans and animals (Tiruneh, 2020).

Ensets are grown exclusively in Ethiopia's highlands and are not widely recognized abroad (Degefa and Dawit, 2018). The domesticated enset plant is grown solely in Ethiopia (Yemata, 2020). Enset can be kept for a lengthy duration of time, both before and after the process, but it is typically harvested twice per year. It can also be collected over numerous years and every day of the year (Egizabiher *et al.*, 2020). The predominant regions in Ethiopia that produce enset are Central, South, and Southwest Ethiopia. Ensets are grown between 1100 and 3000 m overhead sea level, a yearly rainfall of 1100 to 1500 mm, and an average temperature of 10 to 22 degrees Celsius with a relative humidity of 63 to 85% (Ajema, 2022). The typical enset production for a household is 62.5 quintals per hectare. The estimated production of ensets is approximately 0.7 million tons a year on over three million hectares of land in Ethiopia (Haile *et al.*, 2020).

The most common foodstuff products of enset crops are kocho, bulla, and amicho subsequently decortication enset yields fiber as a result of the process (Teshome, 2023). Most fermented starch, commonly referred to as kocho, is made from a mixture of ground corm and decorticated leaf sheaths and is typically consumed with protein-rich foods (Tsegaye and Gizaw, 2015). For a long duration of time, it stores well. Processing and preparation take a long time, and this work is carried out by women (Tiruneh, 2020). It is now being exported from rural to city markets more frequently. In contrast to bulla and kocho, amicho does not demand processing only a part of the inner corm is eaten (Tsegaye and Gizaw, 2015).

The grinding, squeezing, and decorticating of an enset are steps in the size-minimized process. The enset corm grinding is time-wasting and laborious, necessitating technology to manage and make it easier for women during processing (Senbeta *et al.*, 2022). Along with handling daily tasks at home, it is a further duty for women and the workload persists for a lengthy period, which influences sex interactions within a household. The conventional processing methods are complex, laborious, and unhygienic, causing great stress for working women and resulting in a significant loss of grind pulp (Borrell *et al.*, 2020). Grinding enset corms by hand takes two to three hours per the whole root (8 to 15 kg).

In order to address the problem of enset corm grinding, the existing machine had to be significantly modified. The Melkassa Agricultural Engineering research team developed the machine, which had the following shortcomings: The grinding capacity of the existing machine was too low, the efficiency and percentage loss of the machine were also low, the outlet of the grinding machine was not placed in the proper inclination, the hopper of the grinding machine did not have adequate length, the inclined part of the hopper was not positioned in the proper inclination this-forced the users to use wood to push size reduced corm and the length and diameter of the drum were too small. In comparison, the redesigned machine solved the problems related to the existing one also the grinding capacity and efficiency of this machine were high with a low percentage of loss. The modified corm grinding machine primarily differs from conventional methods in that it produces excellent quality and quantity products while requiring less time and labor. There is currently a huge demand for enset by-products as a food source, and this demand is growing significantly, which implies that machine processing is necessary.

The main purposes of grinding corm with a machine are to improve the quality of processed pulp without negotiating the pulp look and to minimize the time requirement for processing. The other purpose of motorized enset corm grinding is to minimize the number of tasks that women must perform while increasing the speed at which corms are processed. Therefore, this study aimed to redesign and evaluate the enset corm grinding machine for enset growers to replace manual grinding with the machine.

MATERIALS and METHODS

Study area

A redesign and testing corm grinding machine was executed at MARC (Figure 1), located near the town of Awash Melkassa, Adama Woreda, East Shewa Zone, Oromia Regional State, 117 km east of Addis Ababa and 17 km southeast of Adama city. It is found at the elevation of 1561 m above sea level and found between 8° 24' 0" to 8° 30' 12" N, 39° 21' 0" to 39° 35' 14" E.

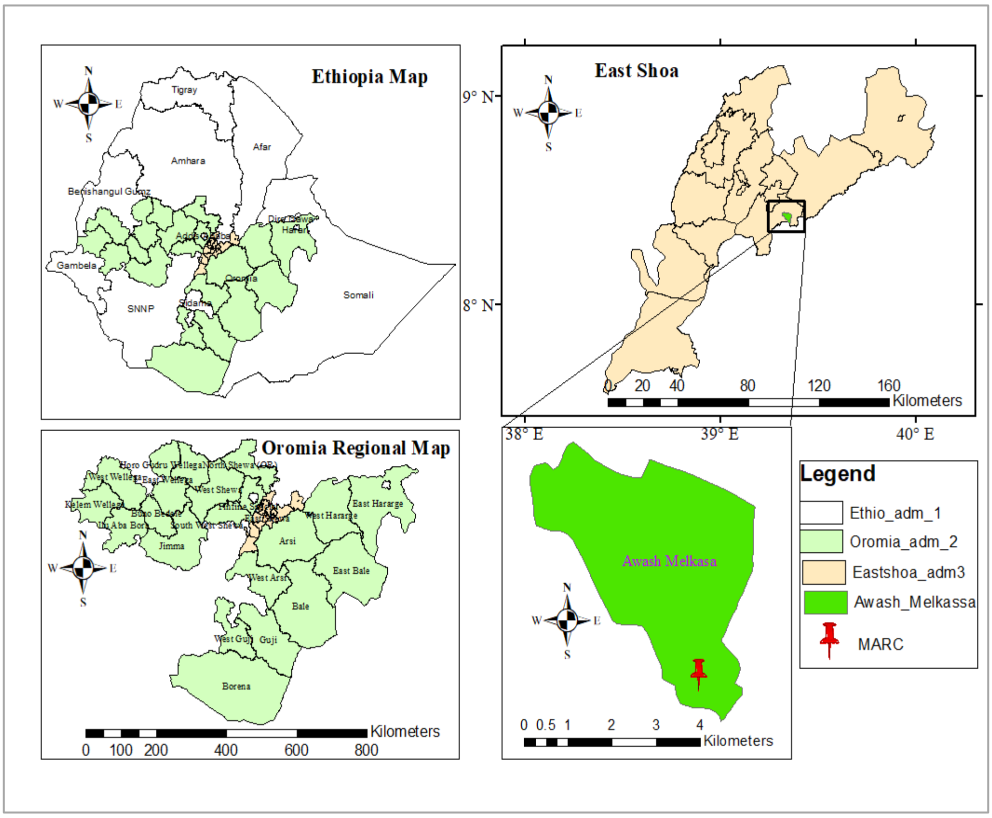

Figure 1. Study area map.

Materials

In this investigation work of simplifying enset processing, a motorized enset corm grinding machine was fabricated as a material that freely existed locally. During the modification of the existing grinding machine, the strengths of the material, as well as the mechanism were considered. The sheet metal, angle iron, shaft, pulleys, belts, bearings, bolts and nuts, and motor had been employed for the improvement of the machine. The digital caliper, tape meter, tachometer, receptacles (sacks), digital weight balance, angle of repose meter, stopwatch and other apparatus were utilized to test the machine's functionality. The test materials were acquired from Oromia, Arsi Zone, and Kulumsa Agricultural Research Centre, to conduct both initial and final assessments on the modified corm grinding machine.

Methods

Redesign considerations

Certain pertinent variables have been taken into account when redesigning the motorized enset corm grinding machine. These variables comprise the need for power, ease with which different parts can be replaced, easiness of movement, safety of part operating, and the cost of maintenance. Since the machine needs mechanical power to run, maintenance would be extremely simple. To achieve optimum function for this machine, proper considerations were made to specify and identify some problems which hindered effective performance as in the former machines, and effort was put to identify the factors and constraints as put together.

Redesign calculation

Hopper redesign

The modified enset corm grinding machine consists of a rectangular-shaped feeding hopper constructed of 2 mm thick aluminum material. The grinder hopper dimensions are 550 mm in length, 450 mm in width, and 450 mm in height. It was fastened to the cover portion and held the enset corm while being grinded. The grinder machine hopper alongside rectangular cross-section had been considered in this case, according to Khurmi and Gupta (2005), the volume of which was obtained using Equation 1 as follows:

$$V = LxWxH \quad (1)$$

Where L is the hopper's length (m), $W$is the hopper's width (m), H is the hopper's height (m), and V is the volume of the hopper (m^3).

Drum redesign

The grinding unit was constructed from a length of 650 mm, diameter of 500 mm, and thickness of 3 mm stainless steel sheet metal that was punctured to form a rough surface on which the grinding is done. The revolving grinding unit produced the constant abrasive force that the rough surface of the grinding unit applied to the enset corm. The grinding unit was powered by an engine motor that was transferred through a V-belt and moved in a circular motion. The shaft that ran through it was backed up by the bearings at each end. The grinding unit was held in place by circular discs on both ends. To guarantee optimal contact between the grinding unit and the enset corm, each grinding surface had a tooth angle of 38°. The grinding unit was cylindrical according to Khurmi and Gupta (2005), the volume of a cylinder, the circumference of the grinding unit, and the force acting on the cylinder unit were determined using Equations 2, 3 and 4 as follows:

$$V=\pi r^2 l \tag{2}$$

$$C=2\pi r \tag{3}$$

$$F=V\rho g \tag{4}$$

Where V is the volume of cylinder (m^3), C is the circumference of the grinding unit (m), F is the force in action on cylinder (N), r is the radius of cylinder (m), l is the length of drum (m), and ρ is the density of stainless steel ($kg\ m^{-3}$).

The grinding force needed by the machine for the enset corm, the power needed to grind the enset corm, and the torque needed to turn the shaft were obtained from Equations 5, 6, 7, and 8 as follows:

$$F=Mt\times g \tag{5}$$

$$P=F\times V \tag{6}$$

$$V=\frac{\pi DN}{60} \tag{7}$$

$$T=F\times r \tag{8}$$

Where Mt is the total mass (kg), P is the power needed to turn the shaft (hp), V is speed ($m\ s^{-1}$), F is force (N), D is the diameter of driver pulley (m), N is the speed of motor (rpm), T is torque (Nm), and r is the radius of driven pulley (m).

Outlet redesign

The outlet was fabricated from aluminum sheet angled at 39.7° and had a thickness of 2 mm. The outlet's inclination angle was connected, but the corm's moisture content determines its inclination of the outlet. The grinder's outlet continued at the frame, which was attached to the cover, its pathways the flow of the grind enset corm into a container as a discharge chute for grinded pulp.

Shaft redesign

The shaft's diameter under varying load conditions can be estimated using Equation 9 (Khurmi and Gupta, 2005). The ASMBE code for shafts that revolve states that when a load has been placed with only a slight amount of shock, the values of Kb = 1.2 to 2 and Kt = 1 to 1.5. Furthermore, it was noted that for the shaft with a keyway, allowable stress τ did not exceed 40 MN m^{-2} (Khurmi and Gupta, 2005).

$$d_s^3=\frac{16}{\pi \tau all}\sqrt{(KbMb)^2+(KtMt)^2} \tag{9}$$

Where d_s is the diameter of the shaft (mm), τall is allowable stress (Nm^{-2}), Mb is the bending moment (Nm), and Mt is the torsional moment (Nm).

Pulley redesign and belt selection

Pulleys are power transmission components; however, their design demands much thought. A pulley's highest pitch diameter and corresponding speeds are indicated by the horsepower rating of the drive pulley. When choosing a belt, careful consideration must be given to the types and dimensions of the standard V-belt as specified by ISO 4184. The shaft speed of the pulley and the speed of the prime mover pulley were related using Equation 10 (Khurmi and Gupta, 2005).

$$\frac{N2}{N1} = \frac{D1}{D2} \tag{10}$$

The nominal pitch length of the belt from a motor shaft to the grinding unit shaft must be determined to know the actual belt size needed to transmit power from a motor to the grinding unit. Then, nominal pitch length and center-to-center distances between pulleys can be determined using Equations 11 and 12 (Khurmi and Gupta 2005).

$$L_b=2Cd+1.57(D2+D1)+\frac{(D2-D1)^2}{4C} \tag{11}$$

$$C_d = \frac{(D1+D2)^2}{2} + D1 \tag{12}$$

Where L_b is length of belt (m) and C_d is distance between driving and driven pulleys (m).

According to Khurmi and Gupta (2005), the wrap angle, angle of the lap, and belt tension for an open belt can be estimated utilizing Equations 13, 14 and 15, respectively.

$$Sin\alpha = \frac{r2-r1}{c} \tag{13}$$

$$\theta = 180 \pm 2sin^{-1}(\frac{D2-D1}{2C}) \tag{14}$$

$$2.3log(\frac{T1}{T2}) = \mu\theta \tag{15}$$

Where α is the wrap angle (°), θ is the angle of lap (rad), and μ is the coefficient of friction.

Description of modified machine

The enset corm grinding machine was powered by a seven-horsepower (7hp) engine motor, which revolves at a constant operating speed. The machine mainly consists of a grinding unit, hopper, drum cover, chute, main frame, shaft, pulley, bearing, V-belt, and engine frame (Table 1). Its grinding mechanism could depend on a grinding unit when the actual grinding operation takes place. The enset corm grinder machine is easy to utilize and less complex to run due to its basic operational mechanism. This modified machine is an outstanding choice for farmers who grow enset. The grinder machine could grind at high grinding up, was fast enough, lasted longer in use with high capacity, and was accessible for farmers.

This modified machine was completely different from the existing one in different ways. The overall length of the machine as indicated (Figure 2) was 720 mm, through a width of 500 mm, besides a height of 1440 mm this indicated that the dimension was higher (Table 2) than existing one. The redesigned machine (Figure 3) was fabricated from stainless steel and aluminum but the existing one was constructed from mild steel. The reason for selecting stainless steel for the fabrication of the grinder was that it had direct contact with the foodstuff to be processed and prevent contamination. The modified machine component was larger than the existing machine (Figure 4) meaning the grinding unit diameter was 500 mm and the grinding unit length was 650 mm for the fabricated machine. For the existing machine, the grinding unit diameter was 200 mm, and the grinding unit length was 300 mm. A redesign of the feeding hopper shape ensures safe feeding and grinding.

Table 1. *Main features or components of both machines.*

No.	Redesigned and existing machine features
1	Feeding hopper
2	Grinding unit or drum
3	Drum cover
4	Chute or outlet
5	Main frame
6	Shaft
7	Pulley
8	Bearing
9	V-belt
10	Engine setting

Table 2. *Bill of materials.*

Item No.	Part number	Descriptions	Quantity
1	Frame width	(40x40x440x3) mm	2
2	Frame length	(40x40x640x3) mm	2
3	Frame height	(40x40x750x3) mm	4
4	Length support	(40x40x570x3) mm	2
5	Width support	(40x40x370x3) mm	2
6	Housing	(640x300xØ440x2) mm	1
7	Shaft	(1000xØ30) mm	1
8	Grating drum	(600xØ40x2) mm	1
9	Circular plate	(Ø40x2) mm	2
10	Discharge chute	(250x370x720x1.5) mm	1
11	Hopper	(40x50x40x1.5) mm	1
12	Bolt and nut	(M16x40) mm	24
13	Rectangular plate	(60x600x3) mm	4
14	Bearing (P206)	(Ø30*x*62x16) mm	2
15	Pulley	(Ø300xØ80x30) mm	1

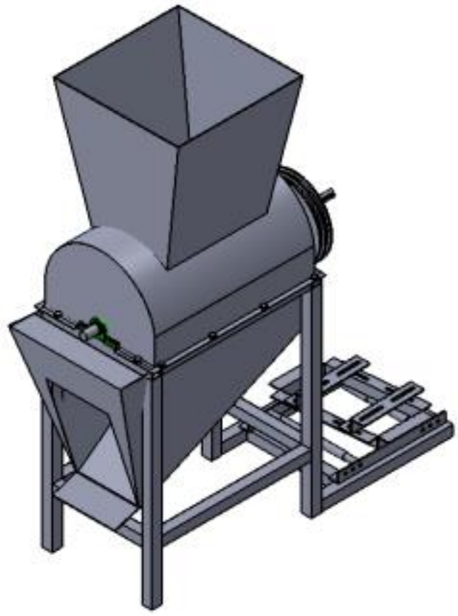

Figure 2. *3D drawing with dimensions for redesigned machine.*

Figure 3. *Redesigned machine.*

Figure 4. *Existing machine.*

Evaluation of the grinding machine

The evaluation was accomplished at an enset corm grinder at three selected operating speeds after weighing the test sample of the enset corm (Figure 6). The grinding was implemented by reducing the size of the peeled enset corm with the help of a knife. The operating speeds were selected based on studies by Kibi (2018), to evaluate the machine's effectiveness on enset corm. Three hundred kilograms (300 kg) of newly removed, clean, and without any harmful enset corm might be utilized in the studies in order to assess the grinder machine.

Evaluation of the grinder machine (Figure 5) was implemented by considering the grinding capacity, grinding efficiency, and loss. Criteria for assessment such as grinding capacity, grinding efficiency, loss, and grinding time were assessed by applying the following equations by Sogaard and Sorensen (2004).

$$\text{Grinding capacity } (kg\ h^{-1}) = \frac{Wc}{t} \quad (16)$$

$$\text{Grinding efficiency } (\%) = \frac{Wc}{Wf} \times 100 \quad (17)$$

$$\text{Percentage loss } = \frac{Wf - Wc}{Wf} \times 100 \quad (18)$$

Where Wc is the weight of collected mash in kilogram, Wf is the weight of corm fed in kilogram, and t is the time taken to grind in hour.

Figure 5. Grinder during testing.

Figure 6. Corms for testing.

Statistical analysis

The experiment implemented a two-factor factorial design within RCBD, and three settings speed through two feed rate levels were considered as treatment combinations. The experiment replicates three times for each treatment. Data analysis was executed using the Statistix 8 software. The significant relationship in factors was indicated using the 95% confidence interval. The comparisons between treatment means were executed by LSD at a 5% level. An analysis of variance

(ANOVA) was executed on the data utilizing a methodology suited to the experiment's design. The two-factor factorial experiment were tested using the ANOVA.

RESULTS and DISCUSSION

The various design parameters were computed, including the power required to grind the enset corm, force required to grind the enset corm, torque needed for the turning shaft and others in order to guarantee the outstanding performance of enset corm grinding machine. The criteria such as compactness, safety, ease of use, maintainability and cost-effectiveness were taken into account. So, the design calculations (Table 3) were carefully undertaken during the redesign of the enset corm grinding machine for meeting the machine's operational needs.

Table 3. *Results for design analysis.*

No.	Design criteria	Computed values	Units
1	Volume of the feeding hopper	0.1114	m^3
2	Volume of the grinding unit	0.0796	m^3
3	Circumference of grinding unit	1.267	m
4	Length of belt	1.18	m
5	Belt speed	7.7	m sec^{-1}
6	Speed ratio	1:3	
7	Belt tension	1958.6	N
8	Lap angle	2.95	rad
9	Distance between pulley	0.14	m
10	Shaft diameter	40	mm
11	Torque	21.41	Nm
12	Grinding power	7	hp
13	Grinding force	298.7	N

The grinder's machine evaluation was executed at three distinct operating speed settings (2000, 2100, and 2200 rpm) as well as the two distinct feeding rate settings (10, and 15 kg min^{-1}) at the moisture content of 56.8% (wet basis) for enset corm about grinding capacity, grinding efficiency, as well as loss. When finished enset corm grinding by the machine, weight measurements were undertaken for the grinding mash, fine mash, course mash, and grinding time. It was observed throughout the evaluation that the machine was producing the greatest amount of output while grinding the enset corm into the mash. The evaluation results revealed that the grinding machine performed incredibly well when it became grinding enset corm. The benefit-cost ratio was calculated to be 1:1.4, indicating that the utilization of the enset corm grinder for grinding corm is an economically feasible choice for enset producers.

Grinding capacity

The ANOVA for a two-factor factorial experiment was executed to test the effects on grinding capacity. The analysis of variance for effects of speed, feed rate as well as interactions for grinder machine grinding capacity was displayed in Table 4. By the findings, an analysis of the variance test illustrated that effects speed, feed rate, as well as interactions had been significant at five percent levels (5%) as shown (Table 4). The findings revealed that feed rate, operating speed, and the combined effects due to both factors affected grinder output.

***Table 4**. An analysis variance of grinding capacity.*

Source	DF	SS	MS	Fo	P	Notice
Replication	2	6.8	3.4			
Operat. speed	2	27485.9	13,742.95	312.48	0.000	Sig.
Feed rate	1	460.3	460.3	10.466	0.008	Sig.
N×Fr	2	599.7	299.85	6.817	0.013	Sig.
Error	10	439.8	43.98			
Total	17	28967.9				

Sig. = significant, Ns = non-significant, P < 0.05, significant at 5 % level, P>0.05, non-significant at 5% level.

The grinding machine's mean grinding capacity varied from 785 to 894.8 kg h^{-1}, as can be seen in Figure 7. With an increase in speed from 2000 to 2200 rpm, the grinding capacity increased from 785 to 894.8 kg h^{-1}. The capacity of the grinder began to rise with an increase in speed but was reduced by the feed rate. This result showed that the operating speed had a direct relation to the grinding capacity as well as adversely related to the feed rate (Kibi, 2018).

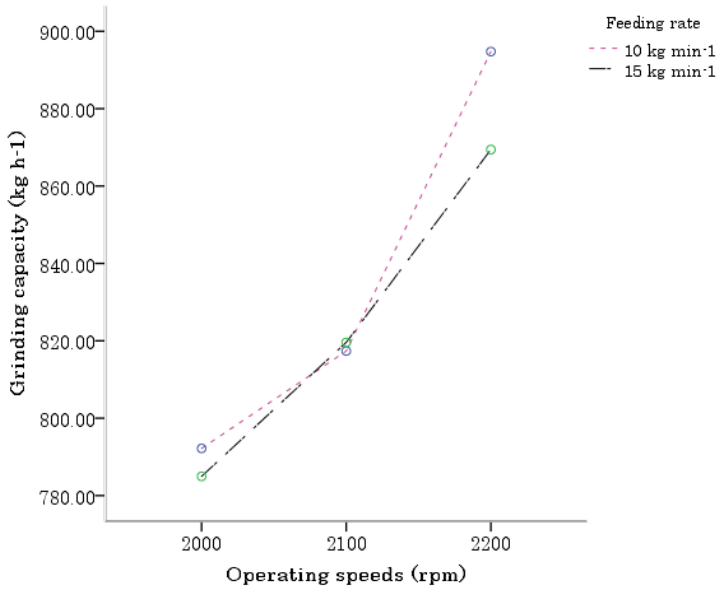

***Figure 7**. Effects of speed as well as feed rate on grinding capacity.*

The findings revealed that the maximum grinding capacity of 894.8 kg h^{-1} was obtained at 2200 rpm operating speed and 10 kg min^{-1} feed rate, while the lowest grinding capacity of 785 kg h^{-1} was obtained at 2000 rpm operating speed and 15 kg min^{-1} feed rate. However, the capacity of the existing machine was obtained as 114.94 kg h^{-1}. This result indicated that in comparison to the modified one, the existing machine's capacity had been extremely low.

Grinding efficiency

The ANOVA for a two-factor factorial experiment was executed to test the effects on grinding efficiency. The analysis of variance for effects of speed, feed rate, as well as interactions on grinder grinding efficiency as can be seen in Table 5. The ANOVA indicated that, at 5% levels, the effects of operating speed and feed rate were significant since p values were below 0.05 ($P < 0.05$). However, their combined impact was non-significant depending on the result obtained (Table 5). From the ANOVA investigation result, machine grinding efficiency was impacted by feed rate and operating speed, not including the combined effects due to both factors.

***Table 5**. An analysis of variance for grinding efficiency.*

Source	DF	SS	MS	Fo	P	Notice
Replication	2	0.0379	0.01895			
Operat. speed	2	42.8926	21.4463	22.993	0.000	Sig.
Feed rate	1	5.4722	5.4722	5.866	0.044	Sig.
N×Fr	2	4.8999	2.44995	2.627	0.144	Ns
Error	10	9.3274	0.93274			
Total	17	51.3963				

Sig. = significant, Ns = non-significant, $P < 0.05$, significant at 5 % level, $P>0.05$, non-significant at 5% level.

Concerning the results, it implied that the mean grinding efficiency varied from 94.3% to 97.9%, as seen in Figure 8. The grinding efficiency ascended from 94.3% to 97.9% as the speeds increased from 2000 to 2200 rpm. As operating speed and feed rate increased, its grinding efficiency also increased. This suggests that the operating speed and feed rate of the material being tested ought to directly influence grinding efficiency.

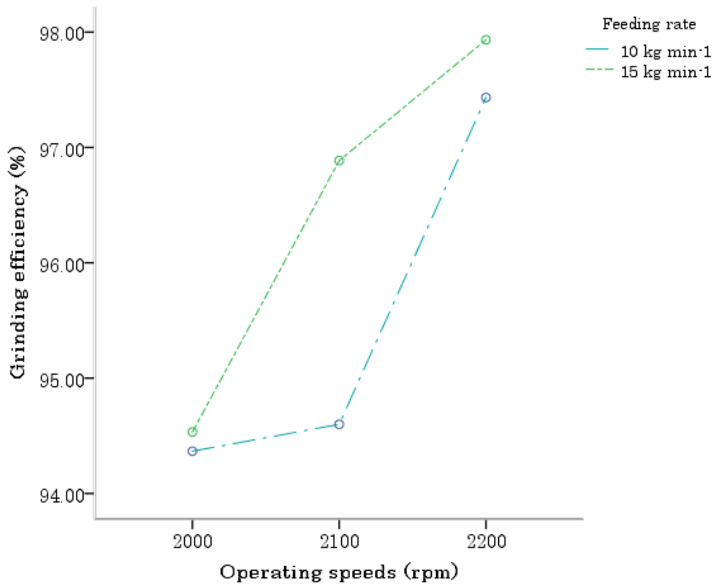

***Figure 8**. Effects of operating speed and feed rate on grinding efficiency.*

The test's results revealed that the maximum grinding efficiency was obtained as 97.9% at 2200 rpm operating speed and 15 kg min^{-1} feed rate, and the minimum grinding efficiency was obtained as 94.3% at 2000 rpm and 10 kg min^{-1} feed rate. The similar trend was reported by Kibi (2018).

Percentage of loss

The ANOVA for a two-factor factorial experiment was executed to test the effects of the loss. The analysis variance effects of speed, feed rate, and interactions on grinder loss can be seen in Table 6. The result of the analysis of variance revealed that, at 5% levels, the effect speed as well as feed rate was significant since p values were below 0.05. However, their combined effect was non-significant depending on the results presented (Table 6). From the results of ANOVA, the machine percentage of loss was affected by feed rate and operating speed except for the combined effects due to both factors.

***Table 6**. An analysis of variance for a percentage of loss.*

Source	DF	SS	MS	Fo	P	Notice
Replication	2	0.0415	0.02075			
Operat. speed	2	42.7548	21.3774	22.99	0.000	Sig.
Feed rate	1	5.5613	5.5613	5.982	0.042	Sig.
N×Fr	2	4.9295	2.46475	2.651	0.142	Ns
Error	10	9.2958	0.92958			
Total	17	52.3385				

Sig. = significant, Ns = non-significant, P < 0.05, significant at 5 % level, P>0.05, non-significant at 5% level.

In accordance with the test findings, the grinder machine's mean percentage of loss varied between 4.1% and 7.7%, as shown in Figure 9. With a rise in speed from 2000 to 2200 rpm, the loss was reduced from 7.7% to 4.1%. The machine's percentage of loss was found to decrease in tandem with a higher feed rate and operating speed. It suggests that there was an adverse association of the percentage of loss with the test material's feed rate and operating speed of the machine.

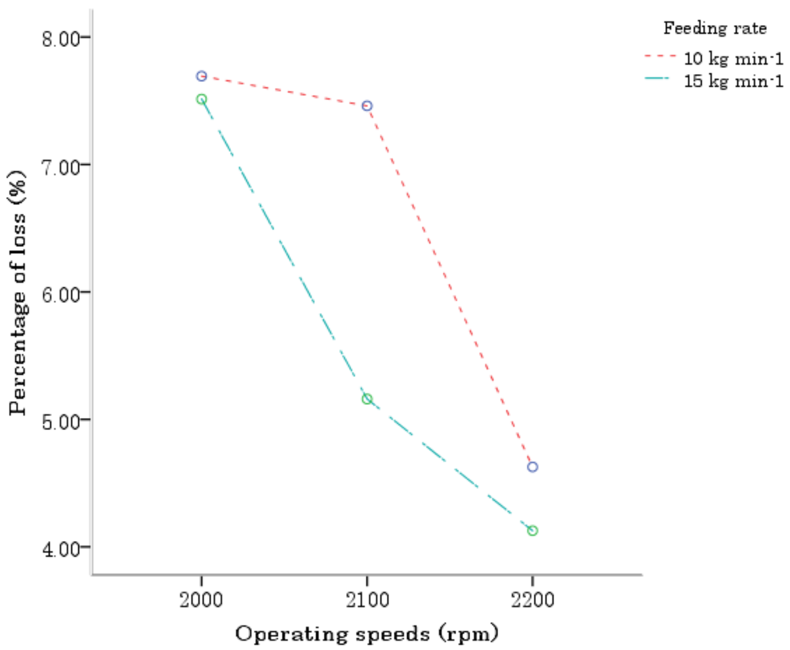

***Figure 9**. Effects of operating speed and feed rate on loss.*

According to test results, the minimum percentage of loss was noted as 4.1% at speed of 2200 rpm and feed rate 15 kg min^{-1}, when operating at 2000 rpm and feed rate of 10 kg min^{-1}, while the highest loss was noted as 7.7%. Considering that the 2200 rpm operating speed had a small percentage of loss when compared to both other speeds.

Grinding time

The ANOVA for a two-factor factorial experiment was executed to test the effects on grinding time. The ANOVA for the effects of speed, feed rate, and interactions on grinder grinding time can be seen in Table 7. The result implied that, at 5% levels of significance, the operating speed and feed rate were significant since p values were smaller than 0.05 ($P < 0.05$). However, their combined effects were not significant based on the results obtained (Table 7). From the results of ANOVA, the machine grinding time was influenced by the feed rate and operating speed, not including the combined effects.

Table 7*. An analysis of variance for grinding time.*

Source	DF	SS	MS	Fo	P	Notice
Replication	2	0.53	0.265			
Operat. speed	2	1791.9	895.95	112.556	0.000	Sig.
Feed rate	1	12361	12361	1,552.8	0.000	Sig.
N×Fr	2	37.4	18.7	2.34924	0.200	Ns
Error	10	79.6	7.96			
Total	17	14047.5				

Sig. = significant, Ns = non-significant, $P < 0.05$, significant at 5 % level, $P>0.05$, non-significant at 5% level.

From the test results, the grinder machine's mean grinding time varied between 186 and 260 seconds at a 2200 rpm operating speed with a feed of 10 kg min^{-1} and at a 2000 rpm operating speed with a feed of 15 kg min^{-1} (Figure 10). Generally, the result shows that there had been a tendency for the machine's grinding time to reduce as it raised the operating speed and lowered the feed rate.

As a result, the grinding time was inversely related to the operating speed but closely related to the feed rate of the enset corm, which was for the reason greater operating speed were executed faster than lower operating speed.

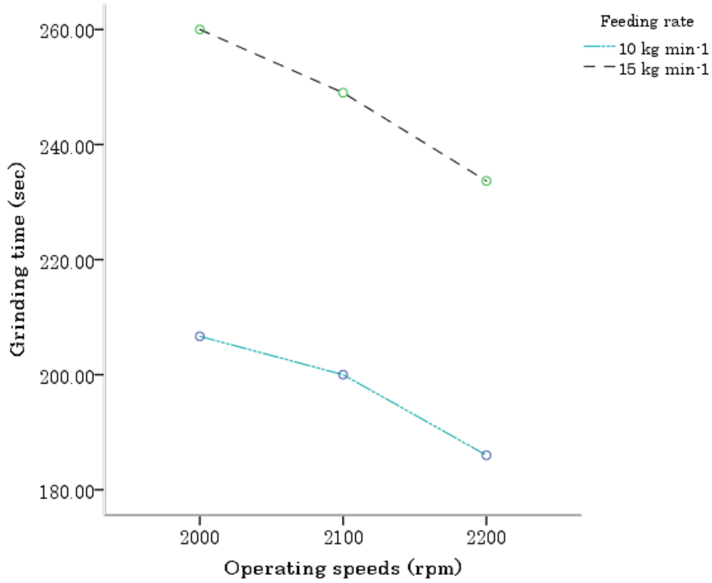

Figure 10. *Effects of operating speed and feed rate on grinding time.*

The lowest grinding time (186 seconds) was achieved with a speed of 2200 rpm and a feed rate of 10 kg min^{-1}, while the highest grinding time (260 seconds) was achieved with a speed of 2000 rpm and a feed rate of 15 kg min^{-1}, contingent on the test results. The grinding time decreased from 260 to 186 seconds at a speed increase of 2000 to 2200 rpm.

Mean separations of treatment means

As given below in Table 8, the LSD all pairwise comparison tests for grinding capacity, grinding efficiency, percentage of loss, and grinding time were carried out for each treatment. In order to identify significant differences between treatment means, this analysis was then exposed to the least significant difference in all pairwise comparison tests for variables for the levels of operating speed and the levels of feed rate. The results of all pairwise comparison tests with the LSD (Table 8) revealed that the treatment means wasn't different at the 5% level. Still, in the case of grinding time, all six means differed from one another at the 5% level.

Table 8. Comparisons between treatment means.

No.	Treatment combination	Operating Speeds (rpm)	Feeding rate (kg min^{-1})	Grinding capacity (kg h^{-1})	Grinding efficiency (%)	Percentage of loss (%)	Grinding time (sec)
1	N1F1	2000	10	792.19^c	94.37^a	7.693^b	206.67^d
2	N1F2	2000	15	784.97^c	94.53^a	7.513^b	260^a
3	N2F1	2100	10	817.37^d	94.6^a	7.46^b	200^e
4	N2F2	2100	15	819.6^d	96.89^b	5.16^a	249^b
5	N3F1	2200	10	894.77^b	97.43^b	4.6267^a	186^f
6	N3F2	2200	15	869.43^a	97.93^b	4.1267^a	233.6^c
7	Grand mean			829.7	96.1	6.09	222.56
8	CV			0.79	0.94	14.85	1.18

N = Speed, F = Feeding rate and CV = Coefficient of variation

CONCLUSION

The grinder's machine evaluation was executed at three distinct operating speed settings (2000, 2100, and 2200 rpm) as well as the two distinct feed rate settings (10 and 15 kg min^{-1}). An investigation has been done on the grinder about grinding capacity, grinding efficiency, percentage of loss, and grinding time. The benefit-cost ratio was calculated to be 1:1.4, indicating that the utilization of the enset corm grinder for grinding corm is an economically feasible choice for enset producers. The results of design calculations implied that the volume of the feeding hopper, volume of the grinding unit, length of the belt, speed ratio, lap angle, shaft diameter, belt tension, torque, the power required to grind, and force required to grind were obtained as 0.1114 m^3, 0.0796 m^3, 1.18 m, 1.3, 2.95 rad, 40 mm, 1958.6 N, 21.41 Nm, 7 hp, and 298.7 N, correspondingly. The results of the assessment revealed that when the speed was raised from 2000 to 2200 rpm, the grinding capacity of the machine raised from 785 to 894.8 kg h^{-1}, whereas the grinding efficiency raised 94.3% toward 97.9%, and loss dropped to 4.1% from 7.7%. The results of all pairwise comparison tests with the LSD indicated that the treatment means weren't different at the 5% level, but in the case of grinding time, means were differing from one another.

DECLARATION OF COMPETING INTEREST

The author declares he have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

The author declared that the following contributions is correct.

Amanuel Erchafo ERTEBO: The author would like to declare that he solely developed all the sections in this manuscript including Investigation, Methodology, Conceptualization, Formal analysis, Data curation, Validation, Writing - original draft, Review, and Editing, Visualization.

ETHICS COMMITTEE DECISION

This article does not require any Ethical Committee Decision.

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Comparison of Village Poultry Activities in Afyonkarahisar and Kütahya Provinces

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ABSTRACT

This study was conducted to determine the current status of village poultry breeding activities in Afyonkarahisar and Kütahya provinces and the important problems encountered in breeding. The study material consisted of 300 survey data from breeders in Afyonkarahisar and Kütahya. According to the research findings, 79.4% and 92% of the breeders in Afyonkarahisar and Kütahya provinces, respectively, were under 60 years of age. 64.7% of the breeders in Afyonkarahisar and 78.7% in Kütahya stated that they preferred domestic breeds, and almost all of them stated that they did not use additional lighting, heating, and ventilation. The main problems in both provinces are disease, feed prices, lack of shelter, and marketing. Providing support to the main problems of breeders through various support and incentive packages can contribute to the sustainability and development of village poultry breeding in the Afyonkarahisar and Kütahya provinces.

Keywords: *Afyonkarahisar, Kütahya, Village poultry, Breeders problems, Solution suggestions*

INTRODUCTION

Türkiye's poultry breeding has significantly advanced in recent years. Cage systems that allow for a higher density of animals per unit area have become prevalent. 90% of intensive layer poultry breeding is done in cage poultry breeding. With this system, egg production reaches 300-310 eggs per hen, while the feed conversion rate is 2.1-2.3 kg (Appleby *et al.*, 1992; Simons, 1997; Sheldon, 2000; Şekeroğlu and Akşimşek, 2009; Müller, 2018; Neves *et al.*, 2021; Karkach, 2024). Village poultry breeding is one of the most important livestock activities to meet the animal protein need in rural areas. Since the extensive breeding system is adopted, egg, and meat yield and hatchability are low and mortality rates are quite high. In addition, inadequate health and protection measures and therefore exposure of animals to diseases are the main problems of the system (Alders and Spradbrow, 2001; Alders and Pym, 2009; Şekeroğlu and Sarıca, 2010). A significant portion of agricultural enterprises in Turkey continue their activities as subsistence family enterprises, but due to failures in economic organization and lack of capital accumulation, enterprises cannot reach commercial size (Keskin *et al.*, 2017). The low level of labor requirement and feeding costs can be considered as its main advantages. Since it does not require special equipment for housing, it has become one of the oldest rearing systems in poultry breeding. Traditional methods have been adopted in village poultry breeding in Turkey. Generally, without any additional feeding, the animal is grazed in the wandering areas to meet its own nutritional needs and is fed with small animals such as insects, worms, etc., and food and bread leftovers. In addition to these, wheat, barley, corn, etc., feeding is done with grain feed and factory feed. Village poultry breeding is divided into three groups as "Traditional Village Poultry Breeding, Advanced Village Poultry Breeding, and Semi-Intensive Village Poultry Breeding" according to breeding methods and animal capacity (Riise *et al.*, 2004). In Türkiye, the average number of animals in traditional village poultry breeding varies between 1-10 animals, in developed village poultry breeding it is 10-50 animals, and in intensive village poultry breeding it varies between 50-200 animals (Şekeroğlu and Sarıca, 2010).

In traditional village poultry breeding, women are generally responsible for the care of the animals, and the hen meat and eggs produced are consumed within the family. In advanced village poultry breeding, all family members take part in the care of the animals, and it is carried out by certain families in rural areas. This system aims to provide additional income from excess domestic consumption. In semi-intensive village poultry breeding, the large number of animals necessitates additional labor and is carried out as a commercial activity. It is not very common in Türkiye and is performed by some families in rural areas (Riise *et al.*, 2004; Güngördü, 2009; Şekeroğlu and Sarıca, 2010).

Village poultry breeding; is also known by different names such as family poultry breeding, garden poultry breeding, and extensive poultry breeding (İnci *et al.*, 2015). Many studies have been carried out in different regions and provinces to determine the structure, characteristics and problems of village poultry breeding in Turkey (Yurt, 2002; Güngördü, 2009; Şekeroğlu and Akşimşek, 2009; Eleroğlu *et al.*, 2014; İnci *et al.*, 2015; İnci *et al.*, 2018; İnci *et al.*, 2019; İnci *et al.*, 2020). According to TUIK's 2023 data, the poultry numbers of the regions in Türkiye are shown in Table 1, and the provincial level animal presence of the Aegean Region is shown in Table 2 (TUIK, 2023).

Traditional village poultry	Advanced village poultry	Semi-intensive village poultry
-Domesticraces	-Domesticrace and culture race	-Hybrids
-High mortality	-Medium	-Low-
-No additional feding	-Free-roaming + additional feed	-Supplementary feeding as needed-
-No vaccination	-Vaccination against Newcastle	-A few vaccinations against illnesses
-No use treatment for diseases	-Rarely treatment	-Complete treatment
-No cage for housing	-Simple structure cage	-With litter floor or cage system
-Egg yield 30-50 pieces egg/hen	-50-150 pieces egg/hen	-250-300 pieces egg/hen
-Weight gain 5-10 g day^{-1}	-10-20 g day^{-1}	-50-55 g day^{-1}

***Table 1.** Numbers of poultry by region in 2022 (TUIK, 2023).*

Region	Poultry Species					
	Broiler	Laying Hen	Turkey	Goose	Duck and Guinea fowl	Total
İstanbul TR1	738.000	793.596	8.986	6.552	5.825	1.552.959
West Marmara TR2	40.777.460	6.250.702	132.409	40.558	62.440	47.263.569
Aegean TR3	63.122.111	37.250.831	1.645.896	84.886	29.891	102.133.615
East Marmara TR4	78.437.668	12.809.256	916.463	53.367	43.758	92.260.512
West Anatolia TR5	11.202.319	15.463.687	54.828	38.992	46.920	26.806.746
Mediterranean TR6	29.426.956	6.330.307	62.686	40.510	52.433	35.912.892
Middle Anatolia TR7	1.486.850	6.655.441	78.512	121.132	29.749	8.371.674
West Black Sea TR8	11.892.379	8.917.462	124.508	102.275	47.684	21.084.308
East Black Sea TR9	129.450	544.350	2.516	10.253	2.162	688.731
Northeast Anatolia TRA	852.764	1.481.206	117.562	690.692	34.301	3.176.525
Middle East Anatolia TRB	12.319.076	3.411.991	126.791	92.447	29.697	15.980.002
Southeast Anatolia TRC	904.766	9.897.498	398.579	103.843	47.597	11.352.283
Total	251.289.799	109.806.327	3.669.726	1.385.507	432.457	366.583.816

***Table 2.** Poultry numbers at provincial level in the Aegean Region for 2022 (TUIK, 2023).*

Provinces	Poultry Species					
	Broiler	Laying Hen	Turkey	Goose	Duck & Guinea fowl	Total
Afyonkarahisar	389.500	14.915.331	41.014	21.407	6.995	15.374.247
Aydın	2.112.260	779.476	24.785	3.032	2.472	2.922.025
Denizli	4.670.186	1.560.157	64.503	5.537	3.039	6.303.422
İzmir	13.210.816	6.644.436	525.798	4.041	3.055	20.388.146
Kütahya	402.000	1.328.194	80.360	33.539	5.292	1.849.385
Manisa	30.361.070	11.278.055	876.216	2.680	1.624	42.519.645
Muğla	0*	516.650	17.126	2.338	4.226	540.340
Uşak	11.976.279	228.532	16.094	12.312	3.188	12.236.405
Total	63.122.111	37.250.831	1.645.89	84.886	29.891	102.133.615

It was announced as "0" by TUIK.

While Marmara accounts for 48% of broiler production and the Aegean Region accounts for 25%, the Aegean Region has a share of 34% and the Marmara Region has a share of 18% in egg poultry production. The Aegean Region also accounts for approximately 45% of Turkey production and 6% of goose production. The poultry presence at the district level of Afyonkarahisar and Kütahya provinces for the last 5 years is shown in Table 3 (TUIK, 2023). Merkez, Başmakçı, Bolvadin and Emirdağ in Afyonkarahisar, and Tavşanlı in Kütahya are the leading districts in egg poultry breeding with one million or more animals.

Table 3. *Poultry numbers of Afyonkarahisar and Kütahya provinces at the district level for the the period 2018-2022 (TUIK, 2023).*

		Poultry Species (Afyonkarahisar Province)									Poultry Species (Kütahya Province)						
Districts	Years	Broiler	L. hen	Turkey	Goose	Duck & Guinea.	Total	+/- %	Dist.	Years	Broiler	L hen	Turkey	Goose	D & G. f.	Total	+/- %
Bayat	2018	0	3935	290	440	335	5000	-	Altıntaş	2018	75000	20000	3574	25062	3341	126977	-
	2019	0	4010	325	420	250	5005	*		2019	75000	20000	3426	25650	3347	254400	100,35
	2020	0	3940	275	445	340	5000	*		2020	150000	19220	3500	24550	2900	454570	78,68
	2021	0	3995	356	483	325	5159	3,18		2021	150500	19275	3550	24555	2915	655365	44,17
	2022	0	4050	425	495	360	5330	3,31		2022	150000	19900	3400	25000	3000	856665	30,72
Başmakçı	2018	0	4210258	12400	756	85	4223499	-	Aslanapa	2018	0	5449	6151	12357	1336	25293	-
	2019	0	3800480	13500	750	83	3814813	*-9,68*		2019	0	5653	6252	11792	1126	50116	98,14
	2020	0	3671049	8705	505	110	3680369	*-3,52*		2020	0	27500	2300	4100	1100	85116	69,84
	2021	0	3023920	8250	480	98	3032748	*-17,60*		2021	0	30000	2500	4500	700	122816	44,29
	2022	0	2751809	14095	110	53	2766067	*-8,79*		2022	0	10000	750	3500	500	137566	12,01
Bolvadin	2018	24000	2261256	1015	2445	980	2289696	-	Domaniç	2018	29500	7000	260	60	40	36860	-
	2019	25000	1717200	985	2100	1055	1746340	*-23,73*		2019	0	7100	14000	80	45	58085	57,58
	2020	25000	1469870	1153	1865	1035	1498923	*-14,17*		2020	0	6600	8650	200	50	73585	26,69
	2021	73000	2674576	998	1902	992	2751468	83,56		2021	0	7000	13600	50	30	94265	28,10
	2022	0	1305935	775	1845	1125	1309680	*-52,40*		2022	0	7000	14120	60	40	115485	22,51
Dazkırı	2018	0	85830	300	205	160	86495	-	Dumlupınar	2018	0	3250	445	450	140	4285	-
	2019	0	73455	321	182	154	74112	*-14,32*		2019	0	3242	400	405	140	8472	97,71
	2020	0	85222	252	181	130	85785	*15,75*		2020	0	335	72	122	0	9001	6,24
	2021	0	77151	226	165	118	77660	*-9,47*		2021	0	330	70	115	0	9516	5,72
	2022	0	1318	67	100	50	1535	*-98,02*		2022	0	335	75	120	0	10046	5,57
Dinar	2018	0	203650	520	1410	510	206090	-	Emet	2018	0	2625	528	183	71	3407	-
	2019	0	673650	510	1110	410	675680	+**		2019	0	2807	531	190	65	7000	105,46
	2020	0	563646	514	1116	414	565690	*-16,28*		2020	0	7115	821	453	180	15569	122,41
	2021	0	563655	490	955	395	565495	*		2021	0	7116	822	454	181	24142	55,06
	2022	0	562650	495	990	390	564525	*		2022	0	7525	821	412	124	33024	36,79

Emirdağ	2018	0	3600	899	361	245	5105	-
	2019	0	3800	925	375	365	5465	7,05
	2020	0	1020960	850	355	250	1022415	+**
	2021	0	1020860	825	315	230	1022230	*
	2022	0	1020760	500	250	200	1021710	*
Evciler ve Kızılören	2018	0	3020	490	585	235	4330	-
	2019	0	2910	455	555	238	4158	*-3,97*
	2020	0	12415	150	410	185	13160	+**
	2021	0	10410	114	335	146	11005	*-16,37*
	2022	0	9244	86	264	127	9721	*-11,67*
Hocalar	2018	0	7000	300	150	50	7500	-
	2019	0	7100	315	158	55	7628	1,70
	2020	0	7600	150	50	20	7820	2,52
	2021	0	8625	470	260	165	9520	21,74
	2022	0	8600	465	250	160	9475	*
Merkez	2018	46000	10187720	6400	8000	2880	10251000	-
	2019	0	7556536	21933	11549	1243	7591261	*-25,95*
	2020	81000	7325600	16300	6700	500	7430100	*-2,12*
	2021	108000	7210200	5400	8500	950	7333050	*-1,30*
	2022	0	7070745	150	1500	100	7072495	*-3,55*
Sandıklı	2018	48500	1034790	1984	1850	590	1087714	-
	2019	49600	1048653	2250	1725	660	1102888	1,40
	2020	61000	970650	712	665	225	1033252	*-6,31*
	2021	63000	974730	715	670	230	1039345	*
	2022	60000	892500	20655	638	203	973996	*-6,29*
Sinanpaşa	2018	122800	335000	4000	4000	2100	467900	-
	2019	122500	334000	3950	3956	2250	466656	*
	2020	97000	401100	4200	4100	2000	508400	8,95
	2021	207300	469200	4250	4200	2100	687050	35,14

Gediz	2018	50700	27000	750	200	190	78840	-
	2019	39500	27500	751	199	192	146982	86,43
	2020	28000	28155	805	200	210	204352	39,03
	2021	40500	41500	1780	620	470	289222	41,53
	2022	80500	10313	625	362	270	381292	31,83
Hisarcık	2018	0	4000	485	60	156	4701	-
	2019	0	1000	400	60	100	6261	33,18
	2020	0	4828	194	57	70	11410	82,82
	2021	0	2500	200	60	80	14250	24,89
	2022	0	2300	180	55	90	16875	18,42
Merkez	2018	166000	13700	41900	2000	855	224455	-
	2019	171500	11100	55500	2050	715	465320	107,31
	2020	177000	31989	58233	1840	840	735222	58,00
	2021	170300	29945	56547	1760	765	994539	35,27
	2022	171500	31624	58778	1843	846	1259130	26,60
Pazarlar	2018	0	1650	40	20	25	1735	-
	2019	0	1700	50	15	20	3520	102,88
	2020	0	360	65	45	8	3998	13,58
	2021	0	365	66	46	7	4482	12,10
	2022	0	370	60	40	5	4957	10,60
Simav	2018	0	19500	315	226	440	20481	-
	2019	0	20250	380	250	415	41776	103,97
	2020	0	21000	450	330	340	63896	52,95
	2021	0	21600	310	280	300	86386	35,20
	2022	0	16500	170	205	185	103446	19,75
Tavşanlı	2018	0	1000000	1213	884	236	1002332	-
	2019	0	1000100	985	850	190	2004458	99,98
	2020	0	948382	747	809	90	2954486	47,40
	2021	0	1084651	713	848	91	4040780	36,76

	2022	101500	508600	680	4395	1890	617065	*-10,18*
Sultandağı	2018	9041	2100	310	315	105	97871	-
	2019	60340	1986	12810	350	103	75589	*-22,77*
	2020	62000	2026	496	841	118	65481	*-13,37*
	2021	101000	2347	562	916	133	104958	60,29
	2022	0	2116	521	1035	117	3789	-**
Çay	2018	0	4100	500	700	500	5800	-
	2019	0	4150	542	725	550	5967	2,88
	2020	0	27283	2013	2005	635	31936	+**
	2021	0	11750	1991	1979	623	16343	*-48,82*
	2022	0	1804	0	300	0	2104	-**
Çobanlar	2018	15000	2500	800	1200	400	19900	-
	2019	15000	2400	600	950	350	19300	*-3,02*
	2020	16000	6500	950	1100	1500	26050	34,97
	2021	30000	8600	1000	1050	1400	42050	61,42
	2022	28000	6500	950	1000	1300	37750	*-10,23*
İhsaniye	2018	146000	763375	800	6805	475	917455	-
	2019	102000	740000	820	6850	500	850170	*-7,33*
	2020	195000	709000	800	6000	520	911320	7,19
	2021	195500	760000	790	7000	500	963790	5,76
	2022	200000	764600	750	7500	550	973400	1,00
İscehisar	2018	0	2100	1750	2800	1120	7770	-
	2019	0	2125	1725	2600	1085	7535	*-3,02*
	2020	0	2300	160	900	900	4260	*-43,46*
	2021	0	2350	150	850	800	4150	*-2,58*
	2022	0	1250	100	350	260	1960	*-52,77*
Şuhut	2018	0	2765	510	512	198	3985	-
	2019	0	2567	240	480	175	3462	*-13,12*
	2020	0	2978	302	505	215	4000	15,54
	2021	0	2950	305	400	120	3775	*-5,63*
	2022	0	2850	300	385	110	3645	*-3,44*

	2022	0	1214677	791	832	99	5257188	30,10
Çavdarhisar	2018	0	2502	450	670	90	3712	-
	2019	0	1520	420	720	80	6452	73,81
	2020	0	1650	460	920	80	9562	48,20
	2021	0	1620	450	985	75	12692	32,73
	2022	0	1650	470	990	100	15902	25,29
Şaphane	2018	0	7665	140	39	29	7873	-
	2019	0	7810	138	60	25	15906	102,03
	2020	0	6900	140	116	35	23097	45,21
	2021	0	5985	123	121	30	29356	27,10
	2022	0	6000	120	120	33	35629	21,37

MATERIALS and METHODS

Ethical Committee of this study was conducted within the scope of the decision of Uşak University Scientific Research and Publication Ethics Committee (protocol code: 2023/04-23 and date: 07 June 2023).

Material

This study was carried out in the villages of Merkez, Sinanpaşa, Bolvadin, Sandıklı, İhsaniye, and İscehisar in Afyonkarahisar province and in Merkez, Dumlupınar, Simav, Altıntaş, Gediz and Aslanapa districts of Kütahya. In Afyonkarahisar, where 150 farmers were reached, including 10 farmers in the Central district and 3 villages each in Sinanpaşa, Bolvadin, Sandıklı, İhsaniye and İscehisar. In Kütahya, according to the same principle, 150 farmers were contacted by reaching 10 villages in the Central district and 3 each in Dumlupınar, Simav, Altıntaş, Gediz and Aslanapa.

Method

A total of 300 breeders in both provinces were interviewed face to face and the survey questions which were answered after the necessary information and approval had been provided. One of the breeders whose answers were sought to questions about the determination of socio-demographic and socio-economic characteristics, reasons for village poultry breeding, livestock activities (Selection of race; domestic race, culture race, mixed flocks) characteristics, diseases seen in hens, treatment application status, precautions, and mortality.

Breeders have stated that local breeds consist of hybrid chickens created by crossing local breeds and cultured breeds that they have been raised by for years. To determine the diseases, questions were asked based on the survey form used by İnci *et al.*, (2015). In the light of the data obtained, it was aimed to compare the village poultry by breeding activities of Afyonkarahisar and Kütahya provinces, to determine the general situation, identify the problems faced by the breeders and offer solutions.

Statistical analysis

The survey forms used in the study were prepared by using previously prepared survey forms on zootechnics and agricultural management. While determining the sample size of the study, a single-stage random probability sampling method grouped based on population proportions was used (İnci *et al.*, 2015). In determining the sample size, the following formula reported by Karasar (1994) used in limited societies, was used.

Formula
n= (z 2 *N*p*q)/(N*d 2 +z 2 *p*q)
n: Sample volume
z: "z" chart value corresponding to 95% significance level
N: Main mass number
p: The probability of the examined event occurring within the population is taken as 50%.
q: The probability that the event under consideration will not occur (1-p).
d: Accepted margin of error (In this study, the margin of error is taken as 5%).

The study aims to present village poultry breeding activities carried out in Afyonkarahisar and Kütahya, the demographic structure and educational status of the breeders, problems related to the sector and solutions to the problems encountered. The data of the study were evaluated in the SPSS 16.0 package program and expressed as descriptive statistics and percentage values.

RESULTS and DISCUSSION

The socio-demographic characteristics of the breeders are shown in Table 4. While it was being determined that 38.7%, 21.3% of the breeders participating in the survey in Afyonkarahisar and Kütahya were men, 61.3%, 78.7% were women, and 90.7% and 86,0% were married, respectively. In both provinces, the percentage of those stating their profession as farmers is 70.7% and 89.3%. It was reported that 75.7% of Bingöl, 84.9% in Muş, 84% in Diyarbakır, 24% in Tekirdağ and 57.6% in Uşak were male (Demirulus *et al.*, 2013; İnci *et al.*, 2015; İnci *et al.*, 2020). In the study conducted in Muş, it was stated that 87.5% of the breeders were married, 49.3% were self-employed, and 59.5% in Bingöl were farmers.

When the age distribution of breeders is examined, the rate of those under the age of 60 is 79.4% in Afyonkarahisar and 92.0% in Kütahya. The results obtained from the age distribution in both provinces are promising in terms of the sustainability of village poultry breeding. While the detected values are higher than the studies conducted in Uşak, Diyarbakır, Bingöl, and Muş (İnci *et al.*, 2015; İnci *et al.*, 2019; İnci *et al.*, 2020; Akın, 2024). In Afyonkarahisar and Kütahya, it was determined that 72.0% and 76.0% of the breeders were primary school graduates, and 65.3% and 77.4% of the households consisted of 1-6 people. While the rate of those who reported being primary school graduates was 67.2% in Uşak and 73.2% in Batman, studies conducted in Muş and Bingöl stated that most of the breeders were illiterate (Güngördü, 2009; Bural, 2015; İnci *et al.* , 2020; Akın, 2024).

Table 4. *Socio-demographic characteristics of breeders.*

				Afyonkarahisar				
Age	**n**	**N.F. (%)**	**Education**	**n**	**N.F. (%)**	**Number of**	**n**	**N.F.**
18-39	37	24.7	Illiterate	8	5.3	1-3	41	27.3
40-59	82	54.7	Primary	108	72.0	4-6	57	38.0
60-80	27	18.0	Secondary	7	4.7	≥7	52	34.7
>80	4	2.7	High	18	12.0	-		
-			University	10	6.0	-		
Gender			**Marital**			**Job**		
Man	58	38.7	Married	136	90.7	Farmer	106	70.7
Woman	92	61.3	Single	14	9.3	Retired	23	15.3
-			-			Self-	21	14.0
Total	**150**	**100**	-	**150**	**100**	-	**150**	**100**
				Kütahya				
18-39	19	12.7	Illiterate	3	2.0	1-3	28	18.7
40-59	119	79.3	Primary	114	76.0	4-6	88	58.7
60-80	12	8.0	Secondary	12	8.0	≥7	34	22.6
>80	0	0	High	17	11.3	-		
-			University	4	2.7	-		
Gender			**Marital**			**Job**		
Man	32	21.3	Married	129	86.0	Farmer	134	89.3
Woman	118	78.7	Single	21	14.0	Retired	12	8.0
-		40-59	-			Self-	4	2.7
Total	**150**	**100**	-	**150**	**100**	-	**150**	**100**

n: Number of families surveyed, N.F: Relative frequency

Table 5 shows the business characteristics of the breeders participating in the survey. The majority of breeders in both provinces prefer domestic breeds (%64.7 %78.7). The rates of those who obtain their animals through natural incubation are %38.0 and %66.0. The obtained values are in line with the studies conducted in Artvin and Batman (Güngördü, 2009; Bayraktar, 2012). 96.3% of the breeders in Tokat, 74.9% in Bingöl and 68% in Batman stated that they obtained their livestock through natural incubation. (Akşimşek, 2008; İnci *et al.*, 2015).

Men generally take part in shelter construction (%72.7 %65.3) and women in maintenance (%78.7, %88.0). It has been determined that animals are generally kept together and soil floor is preferred for their wandering areas.

The study results were found to be compatible with the study conducted in Muş and Uşak, but partially different from the study conducted in Batman (Güngördü, 2009; İnci *et al.*, 2020; Akın, 2024). While less than half of the breeders in both provinces prefer plate-type feeders and drinkers, the percentages of those who state that they do not use feeders and drinkers are %38.7, %58.0 and %23.3 %64.7 respectively. The nesting-box usage rate was determined as %78.0, %81.0. The majority of breeders declared that they do not use additional lighting (%88.0, %94.0), heating (%94.0, %98.0), and ventilation (%96.7, %98.0). Breeders who said they applied disinfection to the hen were determined %61.3, %41.3. The results are in agreement with some studies and partially differ from others (Güngördü, 2009; İnci *et al.*, 2015; İnci *et al.*, 2020; Akın, 2024).

Table 5. *General characteristics of village poultry enterprises.*

Afyonkarahisar

Race	n	N.F. (%)	Animal supply	n	N.F.	Shelter	n	N.F.
Domestic	97	64.7	Market+neighb	54	36.0	Mother	22	14.7
Culture	15	10.0	Natural	57	38.0	Father	109	72.7
Mixed flock	38	25.3	Market+hatchi	39	26.0	All family	19	12.6
Roaming area			**Shelter type**			**Disinfection**		
None	17	11.3	Seperate cage	18	12.0	Not	58	38.7
Soil	122	81.4	Single cage	132	88.0	Lime 1 a	47	31.3
Concrete	11	7.3	-			Lime 2 a	17	11.3
-			-			Other	28	18.7
Care			**Manger type**			**Drinker types**		
Mother	118	78.7	None	58	38.7	None	35	23.3
Father	17	11.3	Plate type	74	49.3	Plate type	92	61.4
Kids	15	10.0	Nipple type	18	12.0	Nipple type	23	15.3
Additional lighting			**Additional heating**			**Additional ventilation**		
None	132	88.0	None	141	94.0	None	145	96.7
Available	18	12.0	Available	9	6.0	Available	5	3.3
Total			-			-		

Kütahya

Race	n	N.F.	Animal supply	n	N.F.	Shelter	n	N.F.
Domestic	118	78.7	Market+neighb	35	23.3	Mother	31	20.7
Culture	7	4.7	Natural	99	66.0	Father	98	65.3
Mixed	25	16.6	Market+hatchi	16	10.7	All family	21	14.0
Roaming area			**Shelter type**			**Disinfection**		
None	32	21.3	Seperate cage	11	7.3	Not	88	58.7
Soil	104	69.4	Single cage	139	92.7	Lime 1 a	32	21.3
Concrete	14	9.3	-			Lime 2 a	22	14.7
-			-			Other	8	5.3
Care			**Manger type**			**Drinker types**		
Mother	132	88.0	None	87	58.0	None	97	64.7
Father	5	3.3	Plate type	53	35.3	Plate type	41	27.3
Kids	13	8.7	Nipple type	10	6.7	Nipple type	12	8.0
Additiona l lighting			**Additional heating**			**Additional ventilation**		
None	141	94.0	None	147	98.0	None	147	98.0
Available	9	6.0	Available	3	2.0	Available	3	2.0
Total			-			-		

n: Number of families surveyed, N.F %: Relative frequency

Table 6. *Nutrition of hens, diseases observed, precautions taken and mortality rate.*

Afyonkarahisar

Feeds	n	N.F. (%)	Feeding time	n	N.F.	Diseases	n	N.F.
Wheat	52	34.6	Morning	43	28.7	Diarrhea	89	67.9
Maize	16	10.7	Night	25	16.7	Newcastle	16	12.2
Barley	10	6.7	Morning-night	72	48.0	Cholera	13	10.0
Mixed	63	42.0	Three meals	10	6.7	Colibacilli	9	6.8
Industrial	9	6.0	-			Hen pox	4	3.1
Precautions			**Period of diseases**			**Death**		
None	125	83.3	Winter	98	65.3	Less than half	124	82.7
Antibiotic	15	10	Spring	13	8.7	Half	17	11.3
Drug-vaccine	10	6.7	Summer	11	7.3	Whole flock	9	6.0
-			Autumn	28	18.7	-		

Kütahya

Feeds	n	N.F.	Feeding time	n	N.F.	Diseases	n	N.F.
Wheat	41	27.3	Morning	17	11.3	Diarrhea	68	55,2
Maize	11	7.3	Night	23	15.4	Newcastle	28	22,8
Barley	4	2.7	Morning-night	105	70.0	Cholera	12	9,8
Mixed	81	54.0	Three meals	5	3.3	Colibacilli	9	7,3
Industrial	13	8.7	-			Hen pox	6	4,9
Precautions			**Period of diseases**			**Death**		
None	117	78.0	Winter	111	74.0	Less than half	113	75,3
Antibiotic	19	12.7	Spring	6	4.0	Half	31	20,7
Drug-	14	9.3	Summer	14	9.3	Whole flock	6	4,0
-			Autumn	19	12.7	-		

Table 6 shows the feed used by breeders in feeding, feeding time, diseases seen in hens, precautions, and mortality rates. Mixed feeding type consisting mostly of grain feeds and bread-food residues has been adopted (%42.0 %54.0). In previous studies, the rate of those using mixed feeding was %60.8 in Uşak, 73.4% in Muş, 36% in Batman, and 34.3% in Tokat, while in the study conducted in Sivas, 89.9% of the breeders stated that they used wheat the most in feeding (Güngördü, 2009; Şekeroğlu and Akşimşek, 2009; Eleroğlu *et al.,* 2014; İnci *et al.,* 2020; Akın, 2024). The percentages of those who stated that they feed animals in the morning and evening are 48.0%, %70.0. In Muş and Bingöl, almost all of the breeders stated that they do the feeding process twice a day, in the morning and evening, and the study findings differed partially from these two studies (İnci et al., 2015; İnci *et al.,* 2020).

While the disease incidence rates in animals are (%87.3) and (%82.0), these diseases include (%67.9) and (%55.2) diarrhea, and (%12.2), and (%22.8) Newcastle disease is in the first place. The rates of those who reported that they could not take any precautions against diseases were %83.3 and %78.0 and it was stated that diseases increased mostly in the winter-autumn periods. %82.7 and %75.3 of breeders stated that less than half of the herd was lost due to diseases. İnci *et al.,* (2020) stated in their study in Muş that 81.7% of breeders had diseases in their animals. 27.5% of the diseases seen are diarrhea and 22% are viral diseases. It was stated that 49.5% of the breeders lost more than half of their animals in winter and 29.4% in autumn. In the study conducted in Bingöl, while the rate of those reporting that all the animals died, were 53.2%, 8.3% stated that less than half of them died, and the study results were found to be different from the two studies (İnci *et al.,* 2015). In studies conducted in Tokat, Akşimşek (2008) stated that 86.3% of breeders applied a treatment method against diseases, while Şekeroğlu and Akşimşek (2009) reported that not all breeders made any vaccinations to protect hens. Güngördü (2009) stated in his study in Batman that 62.9% of the growers and İnci *et al.,* (2015) stated that 75% in Bingöl could not take any precautions against diseases. It was observed that the study results were between the values found in these two studies.

The problems experienced in village poultry breeding and the opinions of breeders about village poultry breeding are shown in Table 7. Breeders stated that the meat and eggs they obtained were not sufficient for themselves and that they provided the remaining parts of their needs from markets (%61.3, %52.0). While %88.0 and %94.0 stated that they did not receive any training throughout the breeding, the percentage of those who stated that the training provided would not be sufficient is %65.3 and %71.8. While the top two main problems were diseases with (%44.0 %29.3) and feed prices with (%30.7, %58.7) other problems were reported as inadequacy of shelters and marketing. The study findings appear to be compatible with the study conducted by İnci *et al.,* (2020) in Muş, and Akın, (2024) in Uşak.

Table 7. *Opinions of breeders about village poultry.*

Afyonkarahisar								
Egg-meat adequacy	**n**	**N.F. (%)**	**Education support status**	**n**	**N.F. (%)**	**Basic issues**	**n**	**N.F. (%)**
Sufficient	58	38.7	No	132	88.0	Disease	66	44.0
Market	92	61.3	Agriculture Dep.	14	9.3	Feed prices	46	30.7
-			University	4	2.7	Shelter	18	12.0
-			-			Marketing	20	13.3
Kütahya								
Egg-meat adequacy	**n**	**N.F. (%)**	**Education support status**	**n**	**N.F. (%)**	**Basic issues**	**n**	**N.F. (%)**
Sufficient	72	48.0	No	141	94.0	Disease	44	29.3
Market	78	52.0	Agriculture Dep.	7	4.7	Feed prices	88	58.7
-			University	2	1.3	Shelter	2	1.3
-			-			Marketing	16	10.7

n: Number of families surveyed, N.F: Relative frequency

CONCLUSION

Afyonkarahisar and Kütahya are among the leading provinces of the Aegean Region in terms of poultry population and are at the top in laying hens breeding. According to data of TUIK (2023), with the production of laying hens approaching 17 million, it covers 43.7% of the region and approximately 14.8% of the total production. Kütahya and Afyonkarahisar account for ¾ of the region in goose production. In both provinces, it has been observed that village poultry breeding is an alternative livestock activity traditionally carried out by farmers in addition to commercial livestock activities. Although the breeders stated that they do village poultry breeding to meet their egg needs, they also reported that the demand for village hen eggs is high and they earn additional income, especially today, when the concept of organic eggs has become widespread. The complaints of the breeders about diseases, feed prices, lack of shelter, marketing of the products and not being able to sell them for their value are remarkable. They reported that they experienced problems regarding the animals harming the environment during the breeding process.

In the light of the information obtained from the study; suggestions have been tried to be stated below in order to solve the problems for a sustainable village poultry breeding in Afyonkarahisar and Kütahya.

Situation/Problem	Suggestions
- Village poultry breeding is done by women in Afyonkarahisar and Kütahya, as is the case throughout Türkiye.	✓ Measures should be taken to prevent women from withdrawing from production. ✓ Educational support programs should be organized by organizing various seminars and training courses on disease, care and nutrition, especially by "Agricultural and Veterinary Faculties of Universities, Provincial and District Directorates of Agriculture".
- Poultry products have sales problems or cannot be sold at their value	✓ Support should be given to breeders to deliver it to the primary consumer without any loss of value. ✓ A cooperation protocol should be drawn up between Municipalities and Provincial/District Directorates of Agriculture and sales points should be offered to breeders.
- Fighting diseases and animal losses	✓ By ensuring adequate employment of veterinarians and health personnel within the Provincial/District Directorates of Agriculture, breeders can be prevented from experiencing animal losses. By providing vaccination and treatment support, infectious diseases can be prevented before they turn into an epidemic.
- Problems remain at the individual level and lack of joint action	✓ Breeders should be encouraged to organize themselves in cooperatives (local, regional and national) and act together to quickly solve common problems.
- Lack of support and incentive packages	✓ Incentive packages should be prepared for the sustainability of village poultry breeding in the annual budgets of the state, and applications to be made through Provincial Directorates of Agriculture and village headmen should be announced to farmers.
- Village poultry breeding remains in the traditional model	✓ Support packages for the transition from the traditional production model of village poultry to advanced village poultry and then to intensive village poultry breeding should be among the policies of the Ministry of Agriculture.

There are a limited number of research and project proposals about the structure, problems and sustainability of village poultry breeding in Turkey. The development of village poultry breeding can enable the family economy and welfare of the rural population to increase.

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DECLARATION OF COMPETING INTEREST

The author declares that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Yuksel AKIN: Declares the contributions to the manuscript such as the following sections: Investigation, methodology, conceptualization, formal analysis, data curation, validation, writing-original draft, review, and editing, visualization.

ETHICS COMMITTEE DECISION

Ethical Committee of this study was conducted within the scope of the decision of Uşak University Scientific Research and Publication Ethics Committee (protocol code: 2023/04-23 and date: 07 June 2023).

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An Investigation into the Physico-Chemical Properties of Abeere (*Hunteria umbellata*) Seed, Seed Oil and Oil Cake

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ABSTRACT

This study investigates the physico-chemical properties of the seeds, seed oil, and oil cake of abeere (Hunteria umbellata), a plant indigenous to certain regions known for its various economic and nutritional benefits. The research focuses on evaluating the physical and chemical characteristics of the seeds oil and cake. Abeere seed oil was extracted using soxhlet extractor with n-hexane as the solvent. The seed oil and seed cake was analyzed for physical properties, proximate and the mineral composition. The results demonstrate that seeds possess a high oil yield of 91% with a light brown and brown colour for the oil and cake respectively. Additionally, the oil cake is shown to be a valuable of protein, making it suitable for animal feed and potential plant-based protein applications. The sodium, iron and the phosphorus content of the seed oil was found to be higher than that of seed cake. Also, a significant difference was observed in the carbohydrate and iron content of the seed oil and seed cake at the p<0.05 level. This comprehensive investigation provides insights into the potential uses of abeere in food, nutrition, and agriculture, highlighting its importance as a sustainable resource in local economies. Further studies are recommended to explore the diverse applications of abeere in the food industry and its impact on food security.

Keywords: *Abeere seed, Seed oil cake, Seed oil, Proximate composition, Mineral composition*

INTRODUCTION

Hunteria umbellata, commonly known as abeere, is a tropical plant belonging to the family Apocynaceae. Its seeds are traditionally used in various cultural cuisines, particularly in West Africa, and are valued for their nutritional properties and potential economic benefits. The seeds contain essential fatty acids, proteins, and other bioactive compounds, making them a source of interest for both nutritional and industrial applications (Afolabi *et al.*, 2018). According to Longe and Momoh (2014), abeere is a good source of minerals, carbohydrates, and protein all essential nutrients for both people and livestock.

The oil extracted from abeere seeds is believed to have physicochemical qualities that can contribute to culinary practices and functional food products. Additionally, the oil cake remaining after oil extraction is often overlooked yet can contain high protein content, making it a valuable resource for animal feed (Akpan *et al.*, 2021). In addition, oil cakes are widely used as organic fertilizers and soil conditioners because of their rich nutrient content, particularly nitrogen, phosphorus, and potassium (Kumar and Singh, 2011), substrate in anaerobic digestion processes in bio gas production (Jain and Tiwari, 2017), extract protein and amino acids for dietary supplements (Rajeshwar, 2015) and production of biochar and soil amendment (Bhattacharya *et al.*, 2019). Understanding the composition and properties of these components can promote sustainable utilization of abeere and contribute to food security in regions where it is cultivated.

Recent studies have explored the physico-chemical properties of various seed oils due to their growing relevance in nutrition and health (Santos *et al.*, 2020). The characterization of seed oils is essential for understanding their suitability for food and industrial uses, influencing aspects such as flavor, shelf life, and health benefits (Farhan *et al.*, 2019). For abeere, comprehensive studies on the seeds, oil, and oil cake remain scarce, highlighting a gap in knowledge regarding its potential applications.

This study aims to investigate the physico-chemical properties of abeere seed oil and oil cake to provide insights into their nutritional. Through this investigation, will contribute to the knowledge base regarding the utilization of this underexplored plant and its products.

MATERIALS and METHODS

Abeere seeds (Figure 1) were procured at the Oja Oba market Oshogbo, Osun State, Nigeria. The seeds were sorted and dehulled by removing the foreign matter and the outer cover of the seeds.

Determination of the abeere seed moisture content

The initial moisture content of the seeds was determined by using 5 g of the sorted seeds and dried using a laboratory oven set at 100ºC for 24 hours. The initial moisture content was determined using Equation 1 wet basis (AOAC, 2009).

$$Moisture\ content = \frac{M_w - M_d}{M_w}\ x\ 100 \qquad (1)$$

Where:

M_w *is the mass of the wet abeere seeds* (g), M_d *is the mass of the dried abree seeds* (g)

Seed oil extraction

Extraction of oils from seeds was carried out following the procedure by Ogunnaike *et al.* (2021). 30 g of milled abeere seeds were wrapped with filter paper and put into a porous thimble of a Soxhlet extractor. This was mounted on a round bottom flask containing 333 ml of n-hexane (solvent). The set-up was then placed on the heating mantle heating at 100ºC. The extraction of oil occurs as the solvent (n-hexane) boils and evaporate condense at the porous thimble where the wrapped paper was placed. The solvent wash down the oil into the flask and the process continues for 8 hours. After this process completed the oil extracted into solvent was separated by re-heating the mixture of oil and n-hexane, the solvent evaporated while the oil was left in the flask. The oil yield extracted from the seed was determined using Equation 2.

$$Oil\ yeild\ (\%) = \frac{Weight\ of\ oil}{Inital\ weight\ of\ samples} x\ 100 \quad (2)$$

Figure 1. *Abeere seeds.*

The colour of the seed oil extracted and seed oil cake was determined using a colour chart. Also, the chemical properties such as the proximate and the mineral component of each extracted seed oil and the seed oil cake (residue material after extraction of oil from the abeere seed) were determined. Each of these was replicated thrice.

Determination ash content of the seed oil cake and extracted seed oil

Each oven-dried sample weighed precisely 2.0 g in powder form before being added to a crucible with a known weight. These were lit in a muffle furnace and heated to 550°C for 8 hours. The ash-containing crucible was then taken out, cooled in a desiccator, weighed, and the ash content was quantified in terms of the sample's oven-dried weight. Equation 3 was used to determine the ash content of the extracted seed oil and the seed oil cake.

$$\%ash\ content = \frac{weight\ of\ \ crucible\ and\ ash - wt.crucible}{weight\ of\ \ crucible\ and\ sample - wt.crucible}\ x\ 100 \quad (3)$$

Determination of moisture content of the seed oil cake and extracted seed oil

10 g of the oil sample was placed into each of the three crucibles after they had been weighed. In an oven set to 105°C, the samples were dried to consistent weights before being chilled in desiccators and weighed. For each sample, the process was carried out three times, and the average value was calculated. The moisture content was determined using Equation 4.

$$Moisture\ content = \frac{Mass\ of\ wet\ oil\ sample - Mass\ of\ the\ dried\ oil\ sample}{Mass\ of\ the\ wet\ oil\ sample}\ x\ 100 \quad (4)$$

Determination of crude fibre of the seed oil cake and extracted seed oil

The samples were separately weighed at 2.0 g into separate beakers, and after stirring, settling, and decanting the samples three times, petroleum ether was used to extract the samples. The samples were then put into a dry 100 ml conical flask after being air dried. At room temperature, 200 cm^3 of a 0.127 M sulphuric acid solution was applied to the samples. The sample was spread out using the first 40 cm^3 of the acid. This was slowly brought to a boil and then cooked for thirty minutes. The sample was filtered to eliminate any insoluble components, and then it was washed with distilled water, 1% HCI, twice ethanol, and finally diethyl ether. Finally, a furnace set at 550°C burned the oven-dried residue. The weight that was left over after measuring the fiber content was ignition and was measured in terms of the sample's weight prior to ignition.

Determination of protein of the seed oil cake and extracted seed oil

By digesting the protein nitrogen in 1g of the dried samples with concentrated H_2SO_4 and in the presence of $CuSO_4$ and Na_2SO_4, the protein nitrogen was transformed into ammonium sulphate. They were heated, and the ammonia that was produced was steam distilled into a solution of boric acid. By titrating the trapped ammonia with 0.1M HCl and Tashirus indicator (double indicator) until a purple pink color was achieved, the nitrogen from the ammonia was determined. The amount of nitrogen that could be calculated was multiplied by the 6.25 mg factor to get the amount of crude protein.

Determination of carbohydrate of the seed oil cake and extracted seed oil

The difference left over after deducting the values for protein, fat, ash, and fiber from the total dry matter was used to calculate the samples' carbohydrate content (AOAC, 2009).

Determination of fat content of the seed oil cake and extracted seed oil

The fat content was determined by extracting the fat from 10 g of the samples using petroleum ether in a soxhlet apparatus. The weight of the fat obtained after evaporating off the petroleum ether from the extract gave the weight of the crude fat in the sample.

Determination of calcium, potassium, and sodium of the seed oil cake and extracted seed oil

Each sample's ash was digested by adding 5 ml of 2 MHCL to the ash in the crucible and heating it until it was completely dry on the heating mantle. A 100 ml volumetric flask has been filled with 5 ml of 2 MHCL that has been heated to boiling and filtered with what man No 1 filter paper. The filtrate was adjusted with distilled water stoppered and prepared for the Jenway Digital Flame Photometer (PFP7 Model) to read the concentration of calcium, potassium, and sodium using the filter corresponding to each mineral element (AOAC, 2009).

Determination of Iron of the seed oil cake and extracted seed oil

The digest of the ash from each of the aforementioned samples as determined by the calcium and potassium test was rinsed with deionized or distilled water and made up to mark in a 100 ml volumetric flask. Through the suction tube, these diluents were sucked into the Buck 211 Atomic Absorption Spectrophotometer (AAS). Using the right fuel and oxidant mixtures, each of the trace mineral elements was read at its specific wavelength using a hollow cathode lamp (AOAC, 2009).

Determination of phosphorus of the seed oil cake and extracted seed oil

Each sample's ash was subjected to the same 2 MHCL treatment as indicated above for the determination of calcium. A 50 ml standard flask was filled to the mark with distilled water, 10 ml of the filter solution and 10 ml of vanadate yellow solution were added, and the flask was shut off and allowed for 10 minutes to allow for complete yellow development. Using a Spectronic 20 spectrophotometer or colorimeter at a wavelength of 470 nm, the optical density (OD) or absorbance of the solution was measured to determine the concentration of phosphorus (AOAC, 2009). The following calculation was used to compute the percentage of phosphorus:

$$\%Phosphorus = \frac{Absorbance\ x\ Slope\ x\ Dilution\ factor}{10000} \quad (5)$$

Data Analysis

All data were analyzed using SPSS software, version 3.6.1 and reported as mean ± standard error. The comparison of obtained data of the samples was done for each of the seed oil and seed oil cake (multiple-comparison) using two ways analysis of variance (ANOVA) with the significance difference at $P<0.05$.

RESULTS and DISCUSSION

Table 1, 2 and 3 shows the physical properties, proximate and the mineral composition of the abeere seed cake and oil respectively. The Analysis of Variance (ANOVA) for the proximate and mineral composition of the abeere seed oil cake and extracted seed oil is as shown in Table 4 and 5 respectively. Table 6 and 7 shows the multiple comparison of proximate and mineral composition of the extracted seed oil and seed oil cake respectively.

Table 1. *Physical properties of abeere seed cake and seed oil.*

Parameter	Colour	Oil Yield
Seed oil cake	Brown	_
Extracted seed oil	Light Brown	9.10%

Table 2. *The proximate composition of the abeere seed oil cake and seed oil.*

S/N	Proximate	Seed oil cake (%)	Extracted seed oil (%)
1	Moisture	7.705	4.607
2	Ash	4.276	2.513
3	Fat	6.649	15.214
4	Fibre	4.202	2.155
5	Protein	15.815	13.538
6	Carbohydrate	61.353	61.974

Table 3. *The mineral composition of abeere seed cake and oil.*

S/N	Minerals	Seed oil cake (%)	Extracted seed oil (%)
1	Sodium(ppm)	71.100	94.750
2	Calcium (ppm)	88.550	81.300
3	Potassium(ppm)	125.000	98.600
4	Iron(ppm)	0.742	0.915
5	Phosphorus(ppm)	90.262	116.128

Table 4. *The analysis of variance (ANOVA) for the proximate of the abeere seed cake and oil.*

Source	Type II Sum of squares	Df	Mean Square	F-value	P-value (P<0.05)
Corrected Model	5164.115[a]	6	860.686	49.210	.000
Intercept	2957.503	1	2957.503	169.097	.000
Parameters	20.562	1	20.562	1.176	.328
Proximate	5143.553	5	1028.711	58.817	.000
Error	87.450	5	17.490		
Total	8209.068	12	860.686		
Corrected Total	5251.565	11			

R^2 = 0 .983

Table 5. Multiple comparisons of the proximate composition of the abeere seed oil cake and seed oil.

Multiple Comparisons

Dependent Variable: Observation
LSD

(I) Proximate	(J) Proximate	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Moisture	Ash	3.0590	2.34847	.222	-2.1737	8.2917
	Fat	-2.8067	2.34847	.260	-8.0394	2.4260
	Fibre	2.7680	2.34847	.266	-2.4647	8.0007
	Protein	-8.5530*	2.34847	.005	-13.7857	-3.3203
	CHO	-53.3320*	2.34847	.000	-58.5647	-48.0993
Ash	moisture	-3.0590	2.34847	.222	-8.2917	2.1737
	Fat	-5.8657*	2.34847	.032	-11.0984	-.6330
	Fibre	-.2910	2.34847	.904	-5.5237	4.9417
	Protein	-11.6120*	2.34847	.001	-16.8447	-6.3793
	CHO	-56.3910*	2.34847	.000	-61.6237	-51.1583
Fat	moisture	2.8067	2.34847	.260	-2.4260	8.0394
	Ash	5.8657*	2.34847	.032	.6330	11.0984
	Fibre	5.5747*	2.34847	.039	.3420	10.8074
	Protein	-5.7463*	2.34847	.034	-10.9790	-.5136
	CHO	-50.5253*	2.34847	.000	-55.7580	-45.2926
Fibre	moisture	-2.7680	2.34847	.266	-8.0007	2.4647
	Ash	.2910	2.34847	.904	-4.9417	5.5237
	Fat	-5.5747*	2.34847	.039	-10.8074	-.3420
	Protein	-11.3210*	2.34847	.001	-16.5537	-6.0883
	CHO	-56.1000*	2.34847	.000	-61.3327	-50.8673
Protein	moisture	8.5530*	2.34847	.005	3.3203	13.7857
	Ash	11.6120*	2.34847	.001	6.3793	16.8447
	Fat	5.7463*	2.34847	.034	.5136	10.9790
	Fibre	11.3210*	2.34847	.001	6.0883	16.5537
	CHO	-44.7790*	2.34847	.000	-50.0117	-39.5463
CHO	moisture	53.3320*	2.34847	.000	48.0993	58.5647
	Ash	56.3910*	2.34847	.000	51.1583	61.6237
	Fat	50.5253*	2.34847	.000	45.2926	55.7580
	Fibre	56.1000*	2.34847	.000	50.8673	61.3327
	Protein	44.7790*	2.34847	.000	39.5463	50.0117

Based on observed means.
The error term is Mean Square(Error) = 8.273.
*. The mean difference is significant at the 0.05 level.

From Table 1 the oil yield and colour of the abeere seed cake and oil were presented. The colour of the abeere seed cake and oil were found to be brown and dark brown respectively. The oil yield from seeds can vary significantly based on the species, extraction methods, and environmental conditions. The oil yield of the abeere seed was found to be 9.10%. The low value of oil yield (9.10%) obtained from this research contradict with the values of the minimum oil yield for abeere seeds can range around 20% (Akpan *et al.*, 2021). However, this value is consistent with lower yield reports for other seeds in the Apocynaceae family or under less optimal extraction conditions (Ogunleye *et al.*, 2020). Additionally, the low oil yield value of 9.10% obtained from abeere seed could be due to species and the extraction method use in this research.

Table 6. *The Analysis of Variance (ANOVA) for the mineral element of the abeere seed cake and seed oil.*

Source	Type II Sum of squares	Df	Mean Square	F-value	P-value
Corrected Model	15629.147a	5	3125.256	13.256	.013
Intercept	59035.711	1	59035.711	250.365	.000
Parameters	22.617	1	22.617	.096	.772
Minerals	15606.530	4	3901.633	16.546	.009
Error	943.195	4	235.799		
Total	75608.054	10			
Corrected Total	16572.343	9			

R^2= 0.943

Table 7. *Multiple comparisons of the mineral element of the abeere seed oil cake and seed oil.*

Multiple Comparisons

Dependent Variable: Observation

LSD

(I) Mineral	(J) Mineral	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Sodium	Calcium	-5.0667	11.43888	.670	-31.4448	21.3114
	Potassium	-37.9833*	11.43888	.011	-64.3614	-11.6052
	Iron	73.4507*	11.43888	.000	47.0726	99.8288
	Phosphorus	-18.1937	11.43888	.150	-44.5718	8.1844
Calcium	Sodium	5.0667	11.43888	.670	-21.3114	31.4448
	Potassium	-32.9167*	11.43888	.021	-59.2948	-6.5386
	Iron	78.5173*	11.43888	.000	52.1392	104.8954
	Phosphorus	-13.1270	11.43888	.284	-39.5051	13.2511
Potassium	Sodium	37.9833*	11.43888	.011	11.6052	64.3614
	Calcium	32.9167*	11.43888	.021	6.5386	59.2948
	Iron	111.4340*	11.43888	.000	85.0559	137.8121
	Phosphorus	19.7897	11.43888	.122	-6.5884	46.1678
Iron	Sodium	-73.4507*	11.43888	.000	-99.8288	-47.0726
	Calcium	-78.5173*	11.43888	.000	-104.8954	-52.1392
	Potassium	-111.4340*	11.43888	.000	-137.8121	-85.0559
	Phosphorus	-91.6443*	11.43888	.000	-118.0224	-65.2662
Phosphorus	Sodium	18.1937	11.43888	.150	-8.1844	44.5718
	Calcium	13.1270	11.43888	.284	-13.2511	39.5051
	Potassium	-19.7897	11.43888	.122	-46.1678	6.5884
	Iron	91.6443*	11.43888	.000	65.2662	118.0224

Based on observed means.
The error term is Mean Square (Error) = 196.272.
*. The mean difference is significant at the 0.05 level.

The moisture content of seeds, seed oil, and oil cake is a crucial parameter that affects their quality, shelf life, and nutritional value. From Table 2, it was discovered that the moisture content of 7.705% and 4.607% was obtained as seed oil cake and seed oil respectively. Nevertheless, the moisture content of the seed oil was found to be high when compared with the findings of Akpan *et al.* (2021) that the moisture content in the extracted seed oil of abeere is generally very low, often less than 0.5%. This implies that the high moisture content of the seed oil will affect the quality and stability of the oil. On the other hand, the moisture content of the seed oil cake was found be in line with the report of Afolabi *et al.* (2018) and Akpan *et al.* (2021) that the moisture content in the oil cake remaining after oil extraction is usually higher, ranging from 6% to 12%. In addition, the ash content from Table 2 of the seedcake (4.276%) was discovered to be higher than that of the seed oil (2.513%). Nonetheless, the value obtained as the ash content of the seed oil cake is in line with the values obtained by Afolabi *et al.* (2018) and Akpan *et al.* (2021). This suggest that mineral composition, which includes essential nutrients that can serve as valuable additives for animal feed or fertilizers. The protein content in seed oil and seed oil cake is a crucial aspect of their nutritional profile, particularly for applications in human nutrition and animal feed (Mardani and Khorasani, 2020). The amount of protein found in the abeere seed oilcake, and seed oil was discovered to be 15.815% and 13.538%, respectively. As shown in Table 2, the protein content (15.815%) of seed oil cake is much higher than seed oil (13.538%). The result is in accordance with that Bello *et al.* (2008). This infers that the availability of such high protein content is beneficial for preserving healthy growth and development in adults, children, and pregnant women, who need a daily intake of high-quality protein.

The fiber content in seed oil and associated seed cakes is an important parameter in evaluating the nutritional and functional properties of the seeds used in oil extraction. According to Akinjogunla *et al.* (2019) and Adebisi and Akinlosotu (2017), the fiber content in seed oil is relatively low, typically ranging from 1.5% to 3.5%, which aligns with the present findings of 2.155% for abeere seed oil. The seed cake, which constitutes the residual material after oil extraction, tends to have a higher fiber content, generally between 3% and 7%, owing to the concentration of lignocellulosic materials (Oboh and Emenike, 2020).

In a study conducted by Okeke and Nwachukwu (2018), it was observed that the fiber content of the seed cake accounted for approximately 4% of the total residual material, which supports the value of 4.202% reported here. The high fiber content in seed cakes indicates their potential as a dietary fiber source and as a raw material for functional food products or bioenergy applications (Adebisi and Akinlosotu, 2017),

In a study by Olaoye and Olaleye (2020), the carbohydrate percentage in seed oils was found to range from 55% to 65%. Similarly, seed cakes, which are the residual materials after oil extraction, often have comparable carbohydrate content, typically around 60% to 65%, depending on the seed type and processing conditions (Adeleke and Adediji, 2018).

In particular, Akinjogunla *et al.* (2019) reported carbohydrate contents of 62% in seed oil and 60% in seed cakes from various oil seeds, indicating the high carbohydrate retention in seed residues. The present findings of 61.974% in seed oil and 61.353% in seed cake are consistent with these reported ranges. Such high carbohydrate levels suggest that residual seed cakes can serve as excellent sources of dietary fiber and polysaccharides, which have potential applications in food industry and bioconversion processes (Oboh and Emenike, 2020).

Minerals are essential for total mental and physical health, and they are also crucial components of bones, teeth, tissues, muscles, blood, and nerve cells (Soetan *et al*., 2010). Generally speaking, they support blood coagulation, neuron responsiveness to physiological stimuli, and acid-base homeostasis. The construction and integrity of cell walls, the maintenance of membrane shape and permeability, the activation of particular enzymes, and the control of a variety of cellular responses to stimuli are all dependent on calcium, according to Ajayi *et al.* (2013). The calcium content of *Hunteria umbellata* seed oil cake was recorded as 88.550 ppm and seed oil as 81.300 ppm. Besides, the low calcium content of both seed oil cake and seed oil obtained acquired is in consistent with the findings by Farhan *et al.* (2019). Additionally, it was shown that heating caused the calcium level to decline following oil extraction (Indrayan *et al.* 2005).

Also, the abeere seed oil was discovered to have a sodium level of 94.750 ppm, which is higher than that of the seed oil cake (71.100 ppm). The use of sodium lowers blood pressure and lowers the chance of developing linked non-communicable diseases. Sodium contributes to the ionic balance of the human body and preserves tissue excitability. Sodium is a key component in the transport of metabolites due to the solubility of salts. *Hunteria umbellata* seed oil cake and seed oil all have potassium content values of 125.000 ppm and 98.600 ppm, respectively. This demonstrates that the cake's potassium content is significantly higher than the 29.52 mg 100 g^{-1} (26.82 ppm) found for *Blighia sapida* (Oyeleke *et al*., 2013). Potassium can be utilized to balance fluid and neuronal transmission, according to (Oyeleke *et al*., 2013) and (Ajayi *et al*., 2013). For the production of hemoglobin, the healthy operation of the central nervous system, and the oxidation of carbohydrates, proteins, and lipids, iron is a crucial trace element (Onawunmi *et al*., 2017). The iron content of *hunteria umbellata* seed oil cake and seed oil reveals that the seed oil has a greater iron component value (0.9 ppm) than the seed oil cake (0.742 ppm). According to Men *et al.* (2021), iron deficiency has a detrimental effect on human health and has been identified by the World Health Organization as one of the most common health issues.

From Table 4, the analysis of variance indicate that carbohydrate content of the seed oil cake and the seed oil has a significant effect on all the other proximate composition. Also, from Table 6, analysis of variance revealed variations in the mineral content of the abeere seed oil and seed oil cake. It was shown that were the iron content has statistically significant influence on the seed oil cake and seed oil at 95% confidence level when compared to other mineral parameters including sodium, calcium, potassium and phosphorus content of the seed oil cake and seed oil.

CONCLUSION

The color taste and odor of the abeere seed oil cake and oil were determined. The oil yield content of the abeere seed was determined as 9.10%. The chemical properties of the abeere seed oil cake and seed oil, such as the minerals and the proximate composition were also determined. The moisture content, fiber, carbohydrate, fat, ash, and protein were found to be the primary components of abeere seed oil cake and seed oil. The analysis of variance revealed that the carbohydrate difference was extremely significant and had an impact on other nearby compositions, demonstrating the high carbohydrate content of abeere seed oil cake and seed oil. Additionally, the sodium, potassium, phosphorus, calcium, and magnesium content of abeere seed oil cake and seed oil were examined. The analysis of variance reveals that iron had a highly significant difference and had an impact on the other mineral composition.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

The author declared that the following contributions is correct.
Aderoju Funmilayo OGUNNAIKE: Investigation of the manuscript, materials and methods used in the research, conceptualization, data analysis and validation and writing of the original draft of the manuscript.
Yetunde Mayowa ADEOSUN: Investigation of the manuscript, materials and methods used in the research, conceptualization, edition of the manuscript and reviewing of the manuscript.
Abidemi Oreoluwa FILANI: Investigation of the manuscript, materials and methods used in the research, conceptualization and data analysis of the original draft of the manuscript.

ETHICS COMMITTEE DECISION

This article does not require any Ethical Committee Decision.

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Inhibition of Quorum Sensing and Biofilm Development by Walnut Rhizosphere Bacteria

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ABSTRACT

Antibiotic resistance is a significant public health threat, and bacterial biofilms may play a role in the development of resistance to antibiotics. Natural biological agents hold promise for solving this problem due to their antibiofilm properties. In this study, extracts of walnut rhizosphere soil bacteria were used to investigate their anti-quorum sensing and antibiofilm characteristics. Four isolates (BT13, BT31, BT39 and BT40) from the walnut rizosphere demonstrated anti-quorum sensing action against Chromobacterium violaceum ATCC 12472 at dosages of 20 mg mL^{-1}. The crude extracts of BT13 (E. mundtii) showed the highest zone diameter, while crude extracts from BT33 did not. The crude extract from the BT39 (E. faecium) isolate, S. aureus (88%), and L. monocytogenes (82%) have the best biofilm inhibition activity. The study identified two walnut rhizosphere bacteria (BT13 and BT39) that exhibited promising antibiofilm and anti-quorum sensing properties, which could serve as natural biological controls against antibiotic-resistant bacteria. In addition, these bacteria may protect both plant and public health by acting as effective antibiotics against resistant pathogenic organisms.

Keywords: *Antibiofilm activity, Antiquorum sensing, Walnut rhizosphere, Enterococcus faecium Enterococcus mundtii*

INTRODUCTION

Antimicrobial resistance (AMR) is a significant global health concern. During the COVID-19 pandemic, the widespread and often inappropriate use of antibiotics has raised serious concerns regarding the acceleration of AMR (Ghosh *et al.,* 2021). Recent studies suggest the need for new natural biological antimicrobial agents to combat the rapidly developing AMR. Diseases caused by pathogenic microorganism, especially in immunocompromised individuals, are significantly influenced by biofilm and quorum sensing (Khan *et al.,* 2021). Numerous illnesses linked to healthcare are caused by bacteria, which also result in longer hospital stays, higher medical expenses, and the development of biofilms on medical equipment (Vestby *et al.,* 2020; Dadi *et al.,* 2021). Despite the use of large amounts of antibiotics, these therapies are not always successful. Therefore, new methods to combat AMR and treatments that can change and eradicate biofilms and quorum sensing are needed. Recent research has shown promising outcomes in natural substances that can suppress quorum sensing and biofilm formation (Hughes and Webber, 2017).

Plant extracts from *Berginia ciliata, Clematis grata,* and *Clematis viticella* have shown anti-biofilm efficacy against *Pseudomonas aeruginosa* PAO1 (Alam *et al.,* 2020). Natural antibiofilms can show anti-quorum sensing and antibiofilm characteristics through naturally occurring bacterial secondary metabolites, and they can also suppress bacterial biofilm formation and quorum sensing activities. Secondary metabolite-producing soil microorganisms have garnered more attention in recent years (Alam *et al.,* 2020).

Examining the antibiofilm and antiquorum sensing characteristics of bacteria from natural settings like soil and water is crucial for developing novel compounds that combat biofilms and resistant infections (Asfour, 2018). Bacterial biofilms, a collection of microorganisms, are resistant to antibacterial treatments and tolerant of adverse conditions. They are responsible for a wide spectrum of chronic diseases, and antibiotic resistance in bacteria induced by biofilms makes diseases more difficult to cure (Donlan, 2002). New approaches, such as anti-quorum sensing detection (QS), are needed to counteract microbial resistance by blocking virulence factors (Roy *et al.,* 2018). QS inhibitors are used to treat chronic resistant bacterial infections and play a vital role in reducing resistance (Miller and Bassler, 2001). Anti-QS controls virulence factor production, bioluminescence emission, binding, motility, competence, biofilm formation, and sporulation, among other physiological processes (Cegelski *et al.,* 2008; Bhardwaj *et al.,* 2013).

Gram-negative bacteria regulate their virulence through a quorum sensing (QS) system, and Quorum Quenching (QQ) bacteria are a sustainable biocontrol method (Ma *et al.,* 2013). However, new medicines are needed to inhibit harmful bacteria that form biofilms and disrupt quorum sensing (Basavaraju *et al.,* 2016).

Research is needed to synthesize novel antimicrobial agents with anti-QS action, such as soil, water, and plant-derived microorganisms. The incidence and diversity of Quorum Quenching bacteria found in walnut rhizosphere soil were studied (Rajput and Bithel 2022; Sabu *et al.,* 2017). Although many studies have been conducted on the anti-quorum sensing and antibiofilm activities of leaf surface (phyllosphere) bacteria of medicinal plants and other plants, the bacteria found in the rhizosphere soil structure of the commercially important walnut tree have not been examined. Therefore, it is crucial to identify new control agents for biological protection against economically significant walnut plant diseases.

Although the economic value of the Walnut plant is high, research on its rhizosphere soil has not yet been done sufficiently. However, a lot of research has concentrated on the anti-quorum sensing and anti-biofilm properties of rhizosphere bacteria, traditional medicinal plants, and other plants. Therefore, finding new biocontrol agents is crucial to preventing diseases that could harm the economically significant walnut plant. This study examined how pathogenic bacteria's quorum sensing and biofilm formation were affected by *E. mundiit and E. faecium* bacteria isolated from walnut soil.

Our objective was to assess these bacterial extracts' suitability for creating novel antimicrobial agents to treat illnesses in people, animals, and plants. Quorum sensing is essential for stopping the development of biofilms and pathogen resistance to antibiotics. Knowing these characteristics could lead to new strategies for the environmentally friendly management of infectious diseases and the control of harmful bacteria. In this direction, researchers are creating novel biocontrol methods that will transform infection control and microbial ecology.

The current study aims to assess the potential applications of bacteria isolated from walnut rhizosphere as novel bactericidal medications for the treatment of illnesses in people, animals, and plants.

The antibiofilm and antiquorum sensing properties of the bacterial isolates were evaluated. These features are essential for preventing dangerous germs to developing resistance to drugs. Therefore, more study is needed to find novel alternatives to antibiotics that will avoid resistant bacteria and ensure the sustainability of renewable therapies.

MATERIALS and METHODS

Collecting soil samples

Rhizosphere soil samples were collected from different walnut growing area of Kırşehir-Kaman (latitude N 39° 22'16″38.51″, longitude E 33° 42′46" 36.16″), at the root depth of young walnut trees. Sampling was carried out under aseptic conditions in the period between August and September 2021.

Bacteria isolated from the rhizosphere of walnuts

In the study, 1 gram of rhizosphere soil was diluted with 10 ml of water to isolate bacteria from the soil sample. The diluted soil samples were plated on nutrient agar plates and incubated at 28°C for 24 to 48 hours. After incubation, colonies with different morphological characteristics were selected and moved onto agar using the streak plate method. Pure isolates were stocked and preserved for identification and analysis (Chikere and Udochukwu, 2014).

The MALDI-TOF-MS automated microbiology system was used to identify the isolates. The presence of these bacteria in the soil was confirmed by the study's identification of 5 bacteria from two species using the MALDI-TOF MS method.

These genera and species are given in Table 1. The anti-quorum sensing experimentation was performed with the violacein producing *Chromobacterium violaceum* ATCC12472. Except for *C. violaceum* ATCC12472, which was incubated at 28°C, all medicinal strains were kept in Tripticase soy agar (TSA) and Tripticase soy broth (TSB) medium at 37°C.

S. aureus ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. aerogenes* ATCC 51342*, L. monocytogenes* ATCC 7644, and *B. cereus* 709 Rome were the pathogenic bacteria used in the anti-biofilm. The agar was then incubated overnight at 37°C overnight.

Identifying strains using MALDI-TOF-MS

MALDI TOF - MS was used to identify the species of strains isolated from walnut rhizosphere soil (Bruker Daltonics, Autoex Speed). This was achieved by identifying mass signals from the most prevalent and conserved genus-, species-, or subgroup-specific ribosomal protein fractions. MALDI-TOF MS log (score) values between 2.000 and 2.299 were considered as stable genus and likely species identifications, whereas values between 2.3 and 3.000 were interpreted as extremely plausible species-level identifications (Alatoom *et al.*, 2011).

The formic acid procedure, as used by Bizzini *et al.* (2010) was used to extract ribosomal protein, and samples were prepared for MS analysis. The samples were moved to the steel target plate using a sterile wood applicator from a single bacterial colony (Ground Steel Target, Bruker Daltonics). The transplanted bacteria were covered with a saturated HCCA matrix (a solution of cyano-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid; CAS Number 28166-41-8). The material is left to dry at room temperature in order to allow for co-crystallization (Bizzini *et al.*, 2010) (Table 1).

Table 1. *Genus and Species Identified by MALDI-TOF MS Method of bacteria isolated from walnut rhizosphere.*

Bacteria code	Organism		MALDI- Biotyper Score Value
	Genus	Species	
BT13	*Enterococcus sp.*	*E. mundtii*	2.443
BT31	*Enterococcus* sp.	*E. faecium*	2.297
BT33	*Enterococcus* sp.	*E. faecium*	2.432
BT39	*Enterococcus* sp.	*E. faecium*	2.366
BT40	*Enterococcus* sp.	*E. faecium*	2.38

Production of crude extract for anti-quorum sensing and anti-biofilm studies

By placing a colony in 5 ml of TSB, 5 rhizosphere isolates were created. For fresh activation, 1 ml of each activated bacteria was added to 100 ml of TSB. After 48-72 hours of incubation at 30°C and 100 rpm in the orbital shaker, the cell-free supernatant from the 100 ml bacteria culture was collected, and after centrifugation at 13800 rpm for 30 minutes, it was diluted with an equivalent volume of ethyl acetate. After that, the crude extract is evaporated in an oven vacuum over night. In order to achieve these final concentrations, 1% dimethyl sulfoxide (DMSO) was added, and the mixture was kept at -20°C. The final concentrations were 5, 10, and 20 mg mL^{-1} stock (w v^{-1}) (Kanagasabhapthy *et al.*, 2009; Younis *et al.*, 2015).

Testing quorum sensing inhibition activity in the crude bacterial extract

Anti-quorum sensing behavior of bacterial extracts against *C. violaceum* was tested using the agar gel diffusion method. On TSA, active *C. violaceum* was spread using a clean cotton swab. Then, 75 µL of extracts at concentrations of 5, 10 and 20 mg mL^{-1} were injected into the opened wells. DMSO, incubated under the same conditions (30°C for 24 hours), was used as a control during the experiment.

It was possible to see anti-quorum sensing activity through a foggy halo field against the background of violeceum pigment. A total of three times this test was run (Abudoleh and Mahasneh, 2017). The plates were then kept at 30°C for a further 24 hours. A translucent condition indicates growth inhibition, while a murky halo (purple hue removal exclusively) is indicating QSI activity (Nithya *et al.*, 2010b).

Violaceum quantitative evaluation

The spectrum of violacein activity by *C. violaceum* (CV12472) in the presence of isolates was investigated by using the Blosser and Gray (2000) method of extracting the violaceum and measuring it. In nutrient broth, bacterial isolates were diluted two-fold, and 50 µl of grown culture (1.7x 10^7CFU / ml) was inoculated and incubated at 28°C until full pigmentation was observed in the blank (untreated culture). To begin, 200 µl of treated and untreated cultures are placed in an Eppendorf tube and lysed by adding 200 µl of 10% SDS, vortexing for 5 seconds, and incubating at room temperature for 5 minutes. Decrease in pigment production in the presence of bacteria isolate measured as percent inhibition = OD of control- OD of treated /OD of control x100 (Khan *et al*, 2009).

Quantification of anti-biofilm activity

For this test, pathogenic bacteria were cultured in Trypticase Soy Broth (TSB) at 37°C for 24 h. 100 µL of crude extract and 100 µL of bacterial cultures were deposited into 96-well polystyrene microtiter plates, and the microplates were then incubated at 37°C for 24 h. Planktonic cells were then discarded. Double distilled water was used to wash the adherent cells and allowed to air dry. After the biofilms were stained with 200 µL of 0.4% (w/v) crystal violet solution, the wells were rinsed twice with clean water. After the wells were air dried, the crystal violet was dissolved in 200 µL of ethanol. The optical density at 595 nm was measured using a microplate reader (Thermo Scientific Microplate Photometer, Multiskan FC, USA). Bacterial cultures without extract were used as controls and BHIB medium was preferred for comparison. There were three runs of this experiment (Theodora *et al.*, 2019).

$$\text{Percentage biofilm inhibition} = \frac{(\text{Control OD595} - \text{Treated OD595}) \times 100\%}{(\text{Control OD595})}$$

Scanning electron microscopy visualization of biofilm inhibition

BT-13 (*E. mundtii*) and BT-39 (*E. feacium*), *S. aureus,* and *E. coli* biofilms were examined by using scanning electron microscopy (SEM). First, biofilms on a glass coverslip were fixed with 2.5% glutaraldehyde for 30 min at 37°C. Coverslips were fixed, followed by three PBS rinses and 15-minute intervals of drying with a graded ethanol solution (30%). The samples were then freeze-dried after reintroducing ethanol using isoamyl acetate. Ultimately, utilizing a scanning electron microscope SEM (FEI Model Quanta FEG 450) study, coverslips were coated with gold. Scanning electron microscopy was used to describe the size, form, and morphology of bacterial antibiofilms. The Yozgat Bozok University Science and Technology Application and Research Center in Yozgat performed SEM (Turkey) (Relucenti *et al.*, 2021).

Finding pathogenic components in the bacterial isolates

It was also established that the bacterial isolates have extracellular enzyme activity for the enzymes lipase, proteinase, hemolysin, and amylase. Using tributyrin plate halo test, lipolytic activity was quantified. This procedure employed 1% tributyrin (v/v) as an enzyme substrate (Kim *et al.*, 2001). On agar plates with skim milk, protease activity is being monitored (Yu *et al.*, 2009). On 1.2% agar plates with an addition of 10% (v/v) sterile skimmed milk, protease generation and proteolytic activity were discovered.

A cleaning zone formed around the colonies as a result of proteolytic strains. On agar-based 5% sheep erythrocytes, hemolytic activity was investigated. Around the colonies, there was a distinct, colorless zone that was a sign of hemolytic activity. A specific substrate was supplied to the solid medium and discovered utilizing a diffusion approach involving developing colonies in order to manufacture extracellular enzymes. At 4 and 28°C, the isolated bacteria were inoculated, and the assays were carried out twice. The open areas surrounding the colonies were quantified in mm as the difference between the halo and the colony's diameter and were thought to be a sign of enzymatic activity (Wang *et al.*, 2007).

RESULTS and DISCUSSION

Anti-quorum sensing and anti-biofilm activity were screened.

The anti-qouroum sensing and anti-biofilm activities of 5 bacteria isolated from 30 rhizosphere soil samples were investigated.

Antiquorum sensing activity detection

Agar well was performed by diffusion method for anti-QS scanning using *C. violaceum* CV12472 strain. The screened 5 soil bacteria at a concentration of 20 mg mL^{-1}, with a pigment inhibition zone of 13.2 and 11.4 mm against CV12472 strain, significant inhibition in pigment production of isolates was detected, respectively (Table 2). Inhibition of pigment production was also detected in bacterial isolates, with a pigment inhibition zone ranging from 11-13 mm against CV12472. No effect on pigment inhibition by bacterial isolates was observed at the tested concentrations of 5 and 10 mg mL^{-1}. Violecein extraction was carried out in cultures of CV12472 treated with soil isolates to prevent the production of violecein.

Table 2. *In disc diffusion assays and pigment inhibition assays, strains have anti-quorum sensing activity.*

Strain No	Origin of isolates	Bacteria	Anti-quorum sensing activity						
			Inhibition zone (mm)			Concentrations (mg mL^{-1})			
			Concentrations (mg mL^{-1})			Violacein inhibition (%)			
			5	10	20	2	4	8	16
BT13	Rhizosphere soil	*E. mundtii*	0	0	13.2	63.3	53.9	23.8	17.8
BT31	Rhizosphere soil	*E. faecium*	0	0	12.3	60.2	45.8	42.0	33.4
BT33	Rhizosphere soil	*E. faecium*	0	0	0	68.3	56.2	58.1	34.5
BT39	Rhizosphere soil	*E. faecium*	0	0	13.1	71.2	61.9	52.8	42.0
BT40	Rhizosphere soil	*E. faecium*	0	0	11.4	72.3	66.7	62.5	56.2

A number of bacteria were isolated as a result of the screening technique utilized in this investigation, ten of which were capable of generating antibacterial substances, and seven of which had both QSI and bacterial growth inhibitory activity against *C. violaceum* ATCC 12472. In order to determine whether Isolates 5 (BT13, BT31, BT33, BT39, and BT40) may produce anti-quorum sensing, additional testing was conducted on them.

Antibiotics therapeutic efficacy is linked to their bactericidal effect, (Lobritz *et al.* 2015) but selective pressure can lead to antibiotic resistance in bacteria (Maeda *et al.* 2012). Conventional therapies are losing effectiveness, and the incidence of antibiotic-resistant pathogenic bacteria is growing uncontrollably (Saga and Yamaguchi 2009). The use of antipathogenic materials to decrease bacterial virulence has become a paradigm for infection control, allowing for no selective pressure on antibiotic resistance development. This approach opens new scientific avenues for developing new therapeutic drugs and addressing the growing issue of antibiotic resistance (Maeda *et al.*, 2012).

Anti-pathogenic materials have emerged as a recent paradigm for bacterial infection control, aiming to decrease virulence rather than destroy the pathogen. This approach does not selectively pressure antibiotic resistance in bacteria and opens up new research avenues for developing new therapeutic drugs, as it does not selectively pressure the growth of antibiotic resistance in bacteria.

Quorun sensing inhibition activity of the bacterial extracts

This study isolated ten bacteria capable of producing antibacterial materials and seven with QSI and bacterial growth inhibitory activity against *C. violaceum* ATCC 12472. Isolates 5 (BT13, BT31, BT33, BT39, and BT40) were tested for anti-quorum generation. The soil from land and beaches was used as a reservoir for isolating microorganisms (Weng *et al.*, 2012). Twelve potentially QSI-active isolates were successfully isolated from the 500 isolates. The oil extract of *Syzygium aromaticum* also showed antibacterial and QSI activity (Khan *et al.*, 2009).

Theodora *et al.* (2019) isolated 11 phyllosphere bacteria with anti-quorum sensing activity. Another study used anti-quroum sensing to separate an actinomycetes strain from soil exposed to Gamma radiation (Lokegaonkar and Nabar, 2017). *B. subtilis* strain R-18 bacterial cell-free culture supernatant was tested against

C. violaceum and *S. marcescens*, demonstrating its quorum sensing inhibitory ability (Devi *et al*., 2018).

The study found that 4 out of 5 rhizosphere isolates can be used as anti-quorum sensing agents due to their ability to thrive in a demanding environment with changing physical conditions and restricted nutrient availability (Hunter *et al*., 2010). The results showed that extracts at concentrations of 5 mg mL^{-1} and 10 mg mL^{-1} had no activity, while activity was being observed at concentrations of 20 mg mL^{-1}. The study also observed inhibition of violet pigments in AHL rhizosphere from *C. violaceum* due to the degradation of metabolites generated by bacteria (Stauff and Bassler, 2011). The bacteria producer and extract concentration also impacted quorum quenching activity (Abudoleh and Mahasneh, 2017) which regulates the gene expression of biofilm-forming cells (Biradar and Devi, 2018; Roy *et al*., 2018).

Future sequencing of rhizosphere bacteria's metabolites could help identify their quorum-quenching agents. Four extracts from walnut rhizosphere showed significant anti-quorum sensing activity against *C. violaceum* CV12472 and anti-biofilm properties against pathogenic bacteria without affecting growth. These extracts showed various results based on biofilm activity quantification in inhibition steps (Table 2). Disrupting autoinducer synthesis, cell-to-cell exchange, autoinducer reception and transduction, and autoinducer degradation can disrupt pathogenic bacteria's quorum sensing mechanism, leading to biofilm inhibition (Grandclement *et al*. 2016; Zhou *et al*., 2017). Biofilm destruction activity may be caused by enzymes or small molecules that hydrolyze biofilm compounds (You *et al*., 2007). Pathogenic bacteria biofilms have different EPS compositions (Gunn *et al*., 2016), and specific enzymes may degrade various EPS compounds (Fleming and Rumbaugh, 2017).

Violacein quantification

The violacein inhibition percentage was 72.3 and 56.2% when *C. violaceum* ATCC 12472 was administered with separate concentrations of extract of BT40 at concentrations of 0.5 and 0.0625 mg mL^{-1} of the bacterial QSI compounds, respectively. This finding is consistent with the findings of Choo *et al*. (2006) who discovered that vanilla extract decreased violacein production by up to 98 %, and Packiavathy *et al*. (2012) who discovered that *Cuminum cyminum* inhibited violacein production by 90%. The extract of *Rosmarinus officinalis* leaves inhibited the synthesis of violacein by 40%, according to Vattem *et al*. (2007).

These findings clearly showed that bacterial QSI compounds can be isolated from natural habitats. Rather than suppressing the pathogen, QS inhibition is an alternative method of pathogen control that involves manipulating gene expression. Bacterial QSI agents would certainly aid in the battle against newly emerged resistant pathogenic bacteria. The area is still in its early stages, and further screening and testing procedures are needed.

Quantification of anti-biofilm agents

The anti-biofilm activity experiment revealed that crude extracts varied in their inhibitory efficacy against all pathogenic bacteria tested, with the greatest results coming from *S. aureus* and *E. coli* and the worst results coming from *L. monocytogenes* and B. *cereus* 709 Roma (Table 3). Pathogens *P. aeruginosa, E. aerogenes and B. cereus* 709 Roma did not show antibiofilm activity. Crude extracts obtained from BT39 (*E. faecium*) isolate, *S. aureus* (88%) and *L. monocytogenes* (82%) had the best biofilm inhibitory activity.

Table 3. *Quantification of anti-biofilm action against pathogenic bacteria*

Pathogens	Activity	BT13	BT31	BT33	BT39	BT40
		E. mundtii	*E. faecium*	*E. faecium*	*E. faecium*	*E. faecium*
S. aureus ATCC 29213	Inhibition	18	0	3	88	3
E. coli ATCC 25922	Inhibition	85	15	15	60	16
P. aeruginosa ATCC 27853	Inhibition	0	0	0	0	0
E. aerogenes ATCC 51342	Inhibition	0	0	0	0	0
L. monocytogenes ATCC 7644	Inhibition	0	0	0	82	0
B. cereus 709 Roma	Inhibition	0	0	0	0	0

Evaluation of anti-biofilm activity

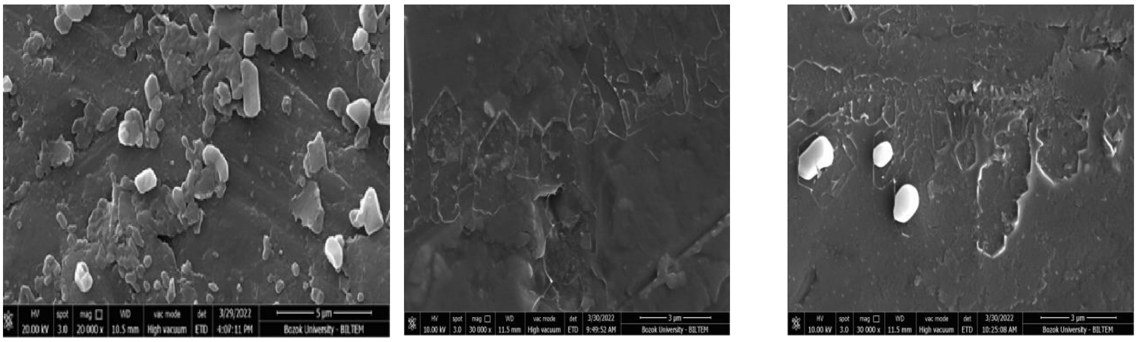
The study found that BT39 isolate extract significantly inhibited biofilm formation without affecting the growth of two standard pathogenic bacteria, *S. aureus* and *L. monocytogenes*. These isolates have a wide spectrum of anti-biofilm function in inhibition ways, and crude extracts of rhizosphere isolates have shown promise as quorum quenching and anti-biofilm agents against some biofilm-forming pathogenic bacteria studied (Table 3). Our results are consistent with previous findings that some marine Bacillus species inhibit *P. aeruginosa* biofilm (Nithya *et al.*, 2010a).

Bacteria prefer biofilm life due to increased resistance to antibacterial agents, which prevents successful treatment of biofilm-associated infections (Høiby *et al.*, 2010). Quorum sensing and biofilm formation are essential for bacteria in social life, and preventing biofilm formation in quorum sensing is crucial for disease treatment (Nadell *et al.*, 2008). Recently, commercially available QQ compounds have been shown to increase the susceptibility of bacterial biofilm to antibiotics, making them suitable for effective treatment of biofilm-associated infections (Brackman *et al.*, 2011).

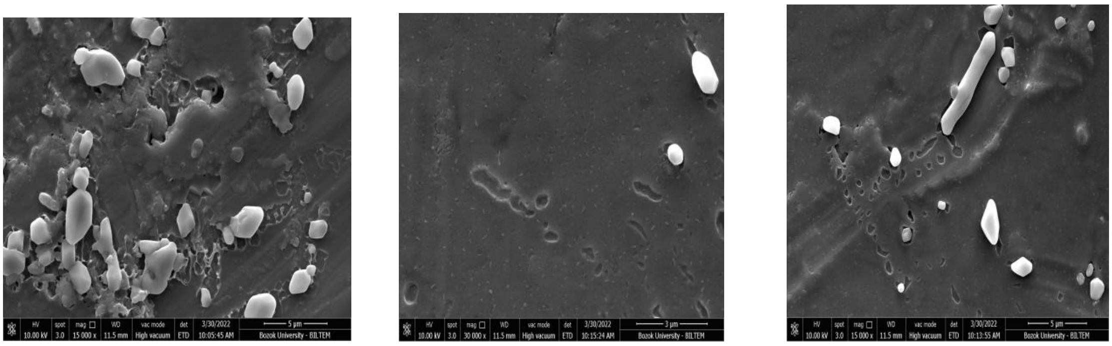
Antibiofilm Activity Evaluation Using Scanning Electron *Microscopy*

The biofilms made up of *E. mundtii* (BT-13), *S. aureus,* and *E. coli* were examined by using and scanning electron microscopy (SEM). First, biofilms on a glass coverslip were fixed with 2.5% glutaraldehyde for 30 min at 37°C. Coverslips were fixed, followed by three PBS rinses and 15-minute intervals of drying with a graded ethanol solution (30%). The samples were then freeze-dried after reintroducing ethanol using isoamyl acetate. Ultimately, utilizing a scanning electron microscope

SEM (FEI Model Quanta FEG 450) study, coverslips were coated with gold. Scanning electron microscopy was used to describe the size, form, and morphology of bacterial antibiofilms (SEM). The Yozgat Bozok University Science and Technology Application and Research Center in Yozgat performed SEM (Turkey).



A) *S. aureus* (Control) B) *E. mundtii* (BT-13) C) *E. faecium* (BT-39)



A) *E. coli* (Control) B) *E. mundtii* (BT-13) C) *E. faecium* (BT-39)

Figure 1. *SEM pictures showing the variations in cell morphology in the biofilm structures of BT-13 and BT-39 bacterial extracts as well as control cells S. aureus and E. coli. (The arrows show that although control cells are intact and tightly encased in the EPS matrix, they are injured and removed from the EPS matrix).*

Evaluation of biofilm inhibition by SEM

SEM was used to detect the biofilm inhibitory activity of bacteria isolated from the walnut rhizosphere against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Visualization using SEM provided additional confirmation of this conclusion (Figure 1). The integrity of the cell walls is compromised, and the multilayer biofilm growth's thickness is reduced. The test bacterial strains also failed to preserve their usual morphology and did not cluster when *P. aeruginosa* was present. The threatened cell walls are to blame for this.

The production of EPS by bacteria, which helps them attach to different surfaces and play a vital role in the growth of microcolonies that result in the creation of biofilms, is another significant feature that has been researched (Leme *et al.* 2006). BT13 and BT39 extracts inhibit a QS system that regulates the release of extracellular DNA needed by the pathogen *E. coli* to form biofilms. These extracts soften the biofilm and make it more susceptible to antibiotics. BT13 and BT39 extracts caused cell death by forming pores in the cell wall of gram-positive bacteria, including pathogen *S. aureus.*

Detection of Virulent Factors of the bacterial isolates

A study was done on the bacterial biofilm supernatants' capacity for hemolysis, lipolysis, and proteolysis. The pathogenicity potential of ten different bacteria was evaluated (Table 4). The size of colonies and haloes was used to gauge enzyme activity. Hemolysis, lipase, and proteinase are used to break down and disperse biofilms. On 5% sheep blood agar plates, biofilm supernatants showed the small-scale and hemolytic activity of hemolytic halos.

These results show that and hemolysin are produced in biofilms and then transferred to the extracellular environment. Larger diameter haloes of proteolysis and lipolysis were noticed in the identical supernatants of tributyrin agar and skim milk agar (Table 3).

Table 4. *Virulent factors of the bacterial isolates measured of diatemer and the colony halo in mm at 4 and 28°C.*

		Temperature (°C)							
		Diameter of virulent factors halo (mm)							
		Lipase		Protease		Hemolysin		Amylase	
Isolates	**Identified species**	4°C	28°C	4°C	28°C	4°C	28°C	4°C	28 °C
BT13	*E. mundtii*	-	-	10.0	15.2	-	-	-	-
BT31	*E. faecium*	-	-	12.2	30.0	-	-	-	-
BT33	*E. faecium*	-	-	12.1	25.0	-	-	-	-
BT39	*E. faecium*	-	-	15.3	25.0	-	-	-	-
BT40	*E. faecium*	-	-	18.2	30.2	-	-	-	-

-No clean zone surrounding the colony + Clear zone surrounding the colony

At 4 and 28°C, *E. faecium* (BT31, BT33, BT39, and BT40) had high protease activity (12.2-30.2 mm), but no lipase, hemolysin, or amylase activity. At 28°C (15.2 mm) and 4°C, *E. mundtii* (BT13) had high protease activity (10.0 mm). The results of present study indicate the difference in the expression of protease by *E. mundtii* (BT13) and *E. faecium* (BT31, BT33, BT39 and BT40) isolates. The results show that rhizosfer isolates in this study displayed proteolytic activity (Table 4).

Detection of Virulent Factors of the bacterial isolates

In this work, bacteria isolated from walnut rhizosphere soil were tested for the presence of virulence factors such lipase, protease, hemolysin, and amylase in specific medium (Abdollahzadeh *et al.,* 2020).

The strains of *E. faecium* (BT31, BT33, BT39, and BT40) and *E. mundtii* (BT13) did not generate lipase, hemolysin, or amylase. However, the clear translucent halo that developed around the colony on the protease-selective media demonstrated that every isolate produced protease.

Despite the fact which this research did not witness the formation of lipase, hemolysin, or amylase enzymes, it has been demonstrated that soil habitats like the rhizosphere of walnuts include bacteria that produce these types of enzymes, which are employed in a variety of uses in industry (Saha *et al.*, 2019). For instance, it has been noted that bacterial amylolytic activities increases within waste soils that include garbage that is abundant in amylase than in garden soils. Food, feed, textile, paper, and biofuel are just a few of the industries that heavily rely on enzymes like pectinase, which break down materials that contain pectin (Abdollahzadeh *et al.*, 2020). Furthermore, bacteria found in the walnut rhizosphere contribute significantly to the cycling of plant nutrients and promote plant health. Four bacteria from the walnut rhizosphere were identified and isolated for this investigation. Gram-negative bacteria taken from comparable soil settings had been shown in earlier research to be useful in biological control against insects in walnut trees, despite the fact that these isolates were Gram-positive. The synthesis, optimisation, and biotechnological potential of the enzymes derived from these isolates require more research.

CONCLUSION

Enterococcus bacteria found in walnut rhizosphere soil can be evaluated in agricultural and medical applications due to their ability to sense pathogenic quorum and prevent biofilm formation. By exploiting these natural antagonistic properties, researchers can develop innovative approaches to combat antibiotic resistance and reduce chronic infections. Future research should focus on understanding the specific molecular interactions and environmental factors that facilitate these beneficial effects and develop solutions against antibiotic-resistant bacteria that pose a threat to public health. These beneficial bacteria can inhibit biofilm formation, reduce virulence, and increase host resistance by producing signaling molecules or enzymes. This sustainable and environmentally friendly approach to antibiotic resistance and infection management requires further research to identify specific bacterial strains or metabolites for therapeutic applications.

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DECLARATION OF COMPETING INTEREST

The authors declare no competing interests.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Belgin ERDEM: Investigation, methodology, conceptualization, validation, writing - original draft and visualization
İlkay AÇIKGÖZ ERKAYA: Formal analysis, data curation, validation and review.
Dilek YALÇIN: Methodology, validation, formal analysis and editing.

ETHICS COMMITTEE DECISION

This article does not require any Ethical Committee Decision.

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Review of Knowledge-Based Management System for Irrigation Scheduling Modeled Upon Reduced Parametric Estimates

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ABSTRACT

Irrigation scheduling is the process of ensuring appropriate, adequate and proportionate crops. Water Management (CWM) stands very important for its water management capability and crop yield optimization among several other advantages. Efficient water management is always crucial for sustainable agricultural practices, traditional irrigation methods often lead to water wastage and suboptimal crop yields Hence, the adoption of technological advancement that spans from the traditional and manual mode to automation, to the application of IOT and extends to the use of Artificial Intelligence (AI). The review paper considers using knowledge-based algorithms for irrigation scheduling, focusing on those that need fewer input parameters. The review looks at several different kinds of knowledge-based algorithms, such as Fuzzy Logic Control, Expert Systems, Neural Networks, Genetic Algorithms, Decision Trees, and Reinforcement learning. The review highlights the fact that knowledge-based algorithms could be a great alternative to traditional irrigation scheduling models, especially when it comes to places where there are few resources for computing power or getting the right data. It also talks about the challenges that come with using these algorithms. Overall, the review makes a strong case for using knowledge-based algorithms for irrigation scheduling. It discusses the tools and techniques used to make these algorithms work well and offers some advice on how to ensure they're being used in the best possible way.

Keywords: *Knowledge-based irrigation algorithms, Sustainable water management, Artificial intelligence in agriculture*

INTRODUCTION

Computational irrigation scheduling methods have been used to optimize water use and crop productivity in agricultural systems. Traditional irrigation scheduling methods are often complex, data-intensive, and computationally demanding, limiting their practical applicability. Research proposes an alternative approach based on knowledge-based algorithms and reduced parametric estimations for irrigation scheduling. The goal is to develop a more efficient and practical method for making irrigation decisions, especially in scenarios where data is scarce. With traditional irrigation methods leading to inefficiency in water use and unnecessary practices, the agricultural sector is becoming increasingly concerned about water scarcity, as mentioned Li *et al.* (2018); Borsato *et al.* (2020). However, advancements in research and technology in Artificial Intelligence (AI), expert systems and in Machine Learning (ML) are offering innovative solutions to address these challenges (Blessy, 2021; Tace *et al.*, 2023; Velmurugan *et al.*, 2024).

Ogidan *et al.* (2019), Ogidan *et al.* (2023) have done some proceeding research work in the irrigation analysis applying the use of FAO Penman-Monteith Equation and extending such to the smart irrigation systems. Smart irrigation systems based on AI and ML algorithms revolutionize water management in agriculture (Abioye *et al.*, 2022). Irrigated agriculture is one of the largest water users in arid and semiarid regions, according to Parmar *et al.* (2019) and Kunapara *et al.* (2016). In addition, the availability of valuable temperature estimate data via remote sensing and geographical information systems plays a significant role in agricultural production, (Parmar *et al.*, 2019). Irrigation with a timer is not the only feature of smart irrigation systems. To maximize and improve irrigation practices, agriculture, and other water-dependent businesses, smart irrigation systems are technologically sophisticated solutions (Talaviya *et al.*, 2020; Obaideen *et al.*, 2022; Vallejo-Gomez *et al.*, 2023). It makes use of intelligent and real-time data. It leverages real-time data and intelligent algorithms to optimize crop irrigation schedules and water delivery. Smart irrigation systems have been determined to ensure that crops receive the precise adequate amount of water precisely when and where it is needed by various factors, including soil moisture levels, weather forecasts, evapotranspiration rates and crop water needs (Obaideen *et al.*, 2022). These systems ensure that water is used more precisely and controlled, leading to benefits such as water conservation, sustainable crop production and reduced operational costs (Ali *et al.*, 2023); (Sharifnasab *et al.*, 2023). Predicting agricultural drought, which leads to substantial yield losses in major crops, is a more challenging than assessing meteorological and hydrological droughts. In order to improve water utilization in irrigation fields, several interconnected components make up the smart irrigation system. The typical IOT-based smart irrigation diagram is shown in Figure 1 below.

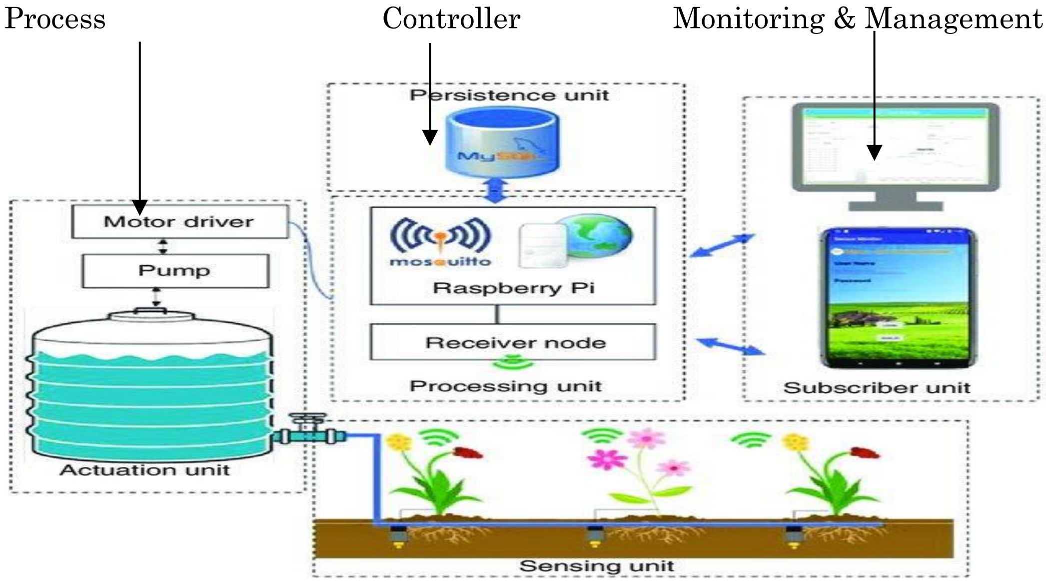

Figure 1. IoT-Based Smart Irrigation System Diagram (Khriji et al., 2021).

Knowledge-Based Algorithms

Knowledge-based algorithms are computer programs designed to imitate human experts' decision-making processes in a precisely defined field, such as irrigation management. These algorithms can effectively consider various variables and decision-making processes to maximize crop output and water utilization. In contrast to traditional process-based models, knowledge-based algorithms require less data and computation power, making them more practical and efficient for irrigation scheduling.

Applications of Knowledge-Based Algorithms in Irrigation Scheduling:

Expert systems are a common application of knowledge-based methods for irrigation scheduling. Expert systems in similar dimensions are computer programs that can simulate the decision-making processes of human specialists in a defined discipline, such as irrigation management. These systems typically consist of an information based (containing domain-specific data) rules, heuristics, and a reasoning process, known as the inference engine, that uses this information to make decisions.

The Decision Support System (DSS), developed by the Food and Agriculture Organization (FAO) of the United Nations, is an example of a knowledge-based irrigation scheduling algorithm. B on FAO Irrigation and Drainage Paper No. 56, this approach provides a comprehensive framework for determining agricultural water requirements and scheduling irrigation. The DSS uses an inference engine to evaluate input data (soil moisture and weather) and apply relevant rules and algorithms to determine the optimal irrigation plan.

Benefits of Knowledge-Based Algorithms

One of the primary benefits of knowledge-based algorithms in irrigation scheduling is that they require less data than complete models. Instead of needing many numbers of parameters that are often difficult to measure or estimate, knowledge-based algorithms only require a small number of parameters, which can be simpler and more readily available. This makes the approach more practical and feasible when data is scarce.

Another advantage is that knowledge-based algorithms are more efficient in computation than fully process-based models. This means that decisions can be made more quickly and with less computational power, making the approach more practical for on-farm implementation.

This research proposes using knowledge-based algorithms and reduced parametric estimations for irrigation scheduling. The proposed approach offers several benefits over traditional methods, including reduced data requirements, increased efficiency of computation, and practical applicability in scarce data scenario. The development and implementation of this method could significantly improve the decision-making process for irrigation scheduling, leading to improved water use efficiency and crop productivity.

LITERATURE REVIEW

Irrigation scheduling is a crucial aspect of agriculture, as it helps optimize water usage and ensure the efficient management of water resources. Knowledge-based algorithms have been widely explored in this domain, leveraging various techniques and approaches to enhance irrigation scheduling decisions. One prominent study by Karimi *et al.* (2021) presents a comprehensive review of knowledge-based algorithms for irrigation scheduling. The authors explore different forms of knowledge-based systems, including fuzzy logic, rule-based systems, and artificial neural networks. They highlight the potential of these algorithms in integrating various factors, such as soil moisture, weather conditions, crop characteristics, and historical data, to provide accurate and context-specific irrigation recommendations. Karimi *et al.* (2021) explores the use of knowledge-based algorithms for irrigation scheduling. It examines various strategies and techniques employed in recent years, highlighting the potential of these algorithms to improve water management in agriculture.

Key Findings

The review identifies several key findings, including

Knowledge-based algorithms can significantly improve irrigation efficiency and water productivity. These algorithms can integrate various data sources, such as soil moisture sensors, weather forecasts, and crop growth models. Different types of knowledge-based algorithms offer varying advantages and limitations, depending on the specific application (Hanyu Wei *et al.*, 2024).

Another study by Brouwer *et al.* (2018) and Gregory *et al.* (2024) focuses on the development of a knowledge-based decision support system in irrigation scheduling. They have proposed a system that combines real-time sensor data, weather forecasts, and expert knowledge to generate irrigation schedules designed to the specific needs of various crops and environments.

In a similar vein, a researcher presents a fuzzy logic-based irrigation scheduling algorithm that incorporates soil moisture, evapotranspiration, and crop water requirements to optimize water usage. Their approach demonstrates the effectiveness of knowledge-based techniques in adapting to variable environmental conditions and achieving water conservation goals, Adel *et al.* (2020). Furthermore, Lozano *et al.* (2020) explore the integration of the processes in machine learning such as support vector machines and artificial neural networks, with knowledge-based approaches for irrigation scheduling. Their findings suggest that the combination of data-driven and knowledge-based techniques can lead to more accurate as well as robust irrigation decisions.

These studies and others in the field have contributed to the growing body of knowledge on the application of knowledge-based algorithms in irrigation scheduling. By leveraging expert knowledge, sensor data, and advanced computational techniques, researchers have developed innovative solutions to address the challenges of efficiency concerns in agricultural water management activities.

Managing waste efficiently is critical to sustainable agriculture, especially in the geographical regions with the problems of water scarcity. Irrigation scheduling, the practice of determining the optimal timing and amount of water to apply to crops, is a key aspect of water management. Legacy irrigation scheduling methods most times rely on manual monitoring and expert judgment, which can be labor-intensive and subject to human error. In recent times, the application of artificial intelligence (AI) techniques has been defined as a promising approach to improve irrigation scheduling.

AI-based Irrigation Sheduling

Researchers have explored various AI methods for irrigation scheduling, including Predictive models: Machine learning models, such as Decision Trees and Artificial Neural Networks, have been used to predict crop water requirements based on factors like weather, soil conditions, and plant characteristics Darouich *et al.* (2017). Optimization algorithms: Optimization techniques, including genetic algorithms and reinforcement learning, have been employed to determine the optimal irrigation strategies which are capable of maximizing crop yield and at the same time minimizing quantity of water usage Deb (2020) and Shiri *et al.* (2017).

Sensor-Based Systems

Ayodele *et al.* (2021) has done a paper review on the application of sensor in an agricultural endeavor in his overview paper for sensor analysis for Health Monitoring, which is based an expert system design for catfish pond. The integration of AI with sensor networks, such as soil moisture sensors and remote sensing data, has enabled real-time monitoring, adaptive irrigation timing and scheduling Taghvaeian *et al.* (2014).

Benefits of Irrigation Scheduling based on AI.

The application of AI to irrigation scheduling has shown several potential benefits: Improved water use efficiency: AI-based models can optimize irrigation schedules reducing water consumption without compromising crop yields Deb *et al.* (2002).

Reduced labor and costs: Automated, AI-driven irrigation systems can reduce the need for manual monitoring and decision-making, thereby lowering labor and operational expenses (Hedley *et al.*, 2009).

Enhanced resilience to climate variability-based systems can adapt to changing environmental conditions, such as drought or excessive rainfall, and provide more accurate and responsive irrigation scheduling Taghvaeian *et al.* (2014).

Increased crop productivity: Optimized irrigation schedules can improve crop health and yield and enhance agricultural productivity (Deb *et al.*, 2002).

Some Applied Studies

Knowledge-based approaches to irrigation scheduling, on the other hand, are integrated with an emphasis on optimizing water use efficiency and maximizing crop yield, considering computational complexities in agro-hydrological systems. The key works have included learning-based and model predictive control frameworks, among which the study of multi-agent MPC for irrigation scheduling was performed by Agyeman *et al.* (2024) using k-means clustering with hydraulic parameter estimates. The system they came up with resulted in 7-23% water savings and increased Intrinsic Water-Use Efficiency (IWUE) by up to 35%, Agyeman *et al.* (2024). In the same direction, model reduction techniques combined with empirical farmer knowledge have made efficient scheduling possible at large hence keeping crops at a non-stressful state with water savings included in the process of Sahoo *et al.* (2022). This contrasts with IoT-based methods that focus on automating irrigation through real-time moisture monitoring, which simplifies operations but lacks the multi-variable optimization seen in Model Prediction Control (MPC) approaches, Jha *et al.* (2023). These methods underscore the importance of parametric simplifications, which are essential for managing large datasets, improving prediction accuracy, and enhancing computational efficiency. Advances in sensor integration, remote sensing, and machine learning further refine these systems to bridge conventional irrigation practices and sustainable water management. Each system brings out the dual goals of sustainability and productivity in tackling the challenges modern agriculture faces. Table 1 below shows insights to some major work in irrigation scheduling and as cited in column 1 of the table.

Table1. *Insights to some major work in irrigation scheduling.*
Source Analysis

No.	Source	Key Insight
1	Learning-based multi-agent MPC for irrigation scheduling (Agyeman *et al.*, 2024)	Demonstrates high efficiency in irrigation water use through advanced multi-agent MPC frameworks.
2	Knowledge-based optimal irrigation scheduling of three-dimensional agro-hydrological systems (Sahoo *et al.*, 2022)	Highlights optimization with reduced dimensionality and empirical knowledge integration.
3	IoT-based irrigation management system (*Jha et al.*, 2023, Mallareddy *et al.*, 2023)	Explores automation in irrigation management using IoT for real-time monitoring.

Challenges and Limitations

While the application of AI to irrigation scheduling shows promise, there still exists several challenges and limitations that require to be addressed: Data availability with the quality: The performances of AI models are highly dependent on data availability and data quality, which can be limited in some agricultural settings.

Interpretability and trust: The complex nature of some AI models, such as deep neural networks, can make it difficult for farmers and stakeholders alike to understand and trust the decision-making process (Hedley *et al.* 2009).

Integration with legacy systems: The integration of AI-based irrigation scheduling with existing farm management systems and infrastructure can be challenging, Taghvaeian *et al.* (2014).

Scalability and deployment: Scaling up AI-based irrigation scheduling from small-scale experiments to large-scale commercial adoption can be a significant hurdle Darouich *et al.* (2017). The application of AI to irrigation scheduling has demonstrated the potential to enhance the water use performance index, labor and cost optimizations, and improve crop productivity. Addressing the challenges and limitations will be crucial for the widespread adoption of AI-based irrigation scheduling in sustainable agriculture.

Advantages of Knowledge-Based Algorithms

Interpretability and Transparency: Knowledge-based algorithms are often more interpretable and transparent than black-box machine learning models. The underlying knowledge base and decision-making process can be explicitly defined, making it easier for users to understand and trust the system's outputs Buchanan and Shortliffe (1984).

Reasoning and Inference: Knowledge-based algorithms can perform complex reasoning and inference based on the rules and relationships stored in the knowledge base. This allows them to handle tasks that require logical thinking, such as troubleshooting, diagnosis, and decision-making.

Domain-Specific Knowledge Incorporation: Knowledge-based algorithms can incorporate and leverage domain-specific knowledge, which can be particularly valuable in specialized or complex domains where expert knowledge is crucial for effective problem-solving Durkin (1994).

Adaptability and Flexibility: Knowledge-based algorithms can be more adaptable and flexible compared to data-driven algorithms, as the knowledge base can be easily updated or modified without retraining the entire system Giarratano and Riley (2005).

Explanation and Justification: Knowledge-based algorithms can provide explanations and justifications for their decisions, which is essential in applications where accountability and transparency are critical, such as in the medical or legal domains Buchanan and Shortliffe (1984).

Handling Uncertainty and Incomplete Information: Knowledge-based algorithms can often handle uncertainty and incomplete information more effectively than data-driven algorithms, by incorporating uncertainty handling mechanisms, such as fuzzy logic or Bayesian reasoning, into the knowledge base Durkin (1994).

Limitations and Challenges

While knowledge-based algorithms offer several advantages, they also face some limitations and challenges:

Knowledge Acquisition and Maintenance: The developing and maintaining a comprehensive knowledge base is often a time-consuming and labor-intensive process, always requiring the involvement of domain experts (Giarratano and Riley, 2005).

Scalability and Performance: Knowledge-based algorithms may not scale as well as data-driven algorithms, particularly for large-scale or high-volume applications, due to the computational overhead associated with reasoning and inference (Durkin, 1994).

Adaptability to New Domains: Transferring knowledge-based algorithms to new domains can be more challenging data-driven approaches, as the knowledge base may need to be significantly modified or rebuilt Buchanan and Shortliffe (1984). Knowledge-based algorithms offer unique advantages, such as interpretability, reasoning capabilities, and the ability to incorporate domain-specific knowledge. While they face some limitations, such as knowledge acquisition and scalability, they remain valuable in applications where transparency, accountability, and domain expertise are crucial. As AI evolves, the synergistic integration of knowledge-based and data-driven approaches can lead to more effective and robust problem-solving solutions.

Literature review on the tools used for the deployment of knowledge-based irrigation algorithms:

The deployment of knowledge-based irrigation algorithms, which rely on expert knowledge and rule-based reasoning to optimize irrigation scheduling, requires various tools and technologies. These tools play a significant role in correct implementation and integration of knowledge-based irrigation systems in agricultural practices.

Tools for Deploying Knowledge-Based Irrigation Algorithms

Expert Systems Shells and Toolkits

Expert system shells and toolkits, such as Jess (Java Expert System Shell), CLIPS (C Language Integrated Production System), and Drools, provide a framework for building and deploying knowledge-based systems. These tools typically include features for knowledge representation, inference engines, and user interfaces, making it easier to develop and deploy knowledge-based irrigation algorithms Giarratano and Riley (2005).

Geographic Information Systems (GIS)

Geographic Information Systems software, such as ArcGIS or QGIS, can be integrated with knowledge-based irrigation algorithms to incorporate spatial data and perform spatial analysis. This allows for integrating factors like soil properties, topography, and microclimate conditions into the decision-making process Shrestha *et al.* (2020).

Sensor Networks and IoT Platforms

Deploying knowledge-based irrigation algorithms often relies on sensor networks and Internet of Things (IoT) platforms to collect real-time data on plant growth, soil moisture and weather conditions. These sensor data can be used to update the knowledge base and adapt the irrigation schedule accordingly Taghvaeian *et al.* (2014).

Decision Support Systems (DSS)

Knowledge-based irrigation algorithms can be integrated into decision support systems (DSS), provide a user-friendly interface for farmers and irrigation managers to access, interpret, and act upon the recommended irrigation schedules. These DSSs often incorporate visualization tools, optimization algorithms, and economic models to support decision-making Darouich *et al.* (2017).

Cloud computing and Web-based Platforms

Cloud computing platforms and web-based applications can facilitate the deployment of knowledge-based irrigation algorithms by providing scalable computing resources, secure data storage, and remote accessibility. This allows for centralized management and distribution of the knowledge-based system to multiple users or locations.

Mobile Applications and Smartphones

Mobile applications and smartphones can serve as an interface for knowledge-based irrigation algorithms, allowing farmers to access and interact with the system directly in the field. These mobile tools can provide real-time recommendations, enable data input, and facilitate communication with the knowledge base Hedley *et al.* (2009).

Challenges and Considerations

Deploying knowledge-based irrigation algorithms requires addressing several challenges and considerations which are explained as follows:

Integration with Existing Farm Infrastructure

Seamless integration of the knowledge-based system with existing farm management systems, irrigation equipment, and data sources is crucial for successful deployment Taghvaeian *et al.* (2014).

User Acceptance and Training

Ensuring user acceptance and providing adequate training for farmers and irrigation managers is essential for successfully adopting knowledge-based irrigation algorithms (Hedley *et al.*, 2009).

Data Quality and Availability

The performance of knowledge-based irrigation algorithms is predominantly dependent on the quality of data; and availability of the input data, requiring careful consideration of sensor calibration, data management, and quality control.

Scalability and Maintenance.

Scaling up the deployment of knowledge-based irrigation algorithms from small-scale pilots to large-scale commercial applications, and maintaining and updating the knowledge base over time, can pose significant challenges Darouich *et al.* (2017). The deployment of knowledge-based irrigation algorithms relies on various tools and technologies, including expert system shells, GIS, sensor networks, decision support systems, cloud computing, and mobile applications. By leveraging these tools, knowledge-based irrigation algorithms can be effectively integrated into agricultural practices, which leads to improved water management and sustainable irrigation implementations. However, addressing the challenges related to integration, user acceptance, data quality, and scalability are necessary for these knowledge-based systems' successful deployment and long-term success.

Use of knowledge-based algorithms for reduced parameter irrigation scheduling:

Irrigation scheduling is a critical aspect of water management for some agricultural processes, as it aims to optimize the timing and amount of water application for maximizing crop yield and minimizing water usage. Traditional irrigation scheduling methods often rely on complex models that require many input parameters, such as soil properties, weather data, and crop characteristics. This can make implementing and adopting these methods challenging, particularly for small-scale farmers with limited resources. Knowledge-based algorithms have been to be a promising approach to addressing this issue by reducing the number of required parameters while still maintaining the effectiveness of irrigation scheduling.

In Knowledge-Based Algorithms for Reduced Parameter Irrigation Scheduling, there are the adoptions of

Rule-based Irrigation Scheduling (RBIS): RBIS rely on a set of expert-derived rules and heuristics to determine the optimal irrigation schedule and amount. These rules can be based on readily available parameters, such as soil moisture, evapotranspiration rates, and crop growth stage, reducing the need for detailed soil and weather data Guo *et al.* (2021), while the Case-Based Reasoning (CBR) scenario could be analyzed with five steps, represented as follows: represent, retrieve, reuse, revise, and retain as shown in figure 2 below, called the R5 CRB model.

Through Data Analysis

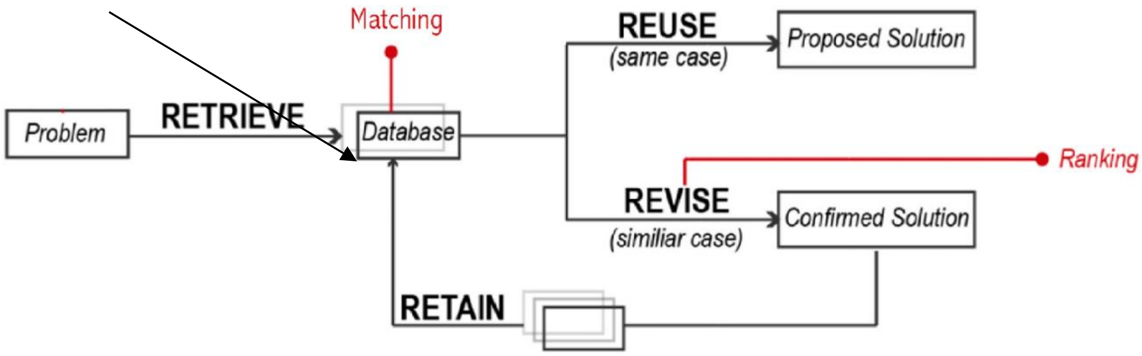

Figure 2. *Diagram For The R-5 model of the CBR approach,* Li *et al.* (2023).

Fuzzy Logic Irrigation Scheduling: Fuzzy logic-based irrigation scheduling algorithms can incorporate expert knowledge and linguistic rules to make irrigation decisions. Using fuzzy membership functions and inference rules, these algorithms can handle imprecise or uncertain data, such as soil moisture levels or weather forecasts, without requiring precise numerical inputs (Benzaouia *et al.*, 2023).

Adaptive Neuro-Fuzzy Inference Systems (ANFIS): The ANFIS integrates the advantages of fuzzy logic and artificial neural networks to create a hybrid knowledge-based algorithm. ANFIS can learn from available data to fine-tune the fuzzy rules and membership functions while allowing expert knowledge integration. This approach can reduce the required parameters compared to traditional irrigation scheduling models (Rouhani *et al.*, 2012).

Case-Based Reasoning (CBR)-based irrigation scheduling systems store and retrieve past successful irrigation strategies as cases, which can then be adapted to the current situation. This approach allows for the incorporation of expert knowledge system and experience without the requirement for a comprehensive numerical model, Zhaoyu *et al.* (2020).

Hybrid Approaches: Researchers have explored integrating knowledge-based algorithms with other techniques, such as sensor-based monitoring and optimization methods, to reduce the parameter requirements further while maintaining the effectiveness of the irrigation scheduling. For example, combining rule-based systems with soil moisture sensors or integrating fuzzy logic with genetic algorithms can lead to more efficient and adaptive irrigation scheduling Darouich *et al.* (2017); Shiri *et al.* (2017).

Benefits and Challenges

The use of knowledge-based algorithms for reduced parameter irrigation scheduling offers several potential benefits:

Reduced data requirements: By relying on smaller input parameters, knowledge-based algorithms can be more accessible and easier to implement, especially for small-scale farmers.

Improved decision-making: The incorporation of expert knowledge and reasoning can lead to more informed and context-specific irrigation decisions.

Adaptability and flexibility: Knowledge-based algorithms can be more easily updated and adapted to changing conditions than complex numerical models.

However, there are also challenges associated with the deployment of knowledge-based irrigation scheduling algorithms:

Knowledge acquisition and representation: Capturing and formalizing expert knowledge into a usable knowledge base can be a time-consuming and labor-intensive.

Validation and testing: Ensuring the accuracy and reliability of knowledge-based algorithms in real-world agricultural settings can be challenging.

Integration with existing systems: Seamless integration of knowledge-based irrigation scheduling with other farm management tools and infrastructure is crucial for widespread adoption.

CONCLUSION

The use of knowledge-based algorithms for reduced parameter irrigation scheduling shows promises in addressing the challenges of traditional irrigation scheduling models, particularly in terms of data requirements and accessibility. By leveraging expert knowledge, fuzzy logic, and adaptive learning, these algorithms can provide effective irrigation scheduling solutions while reducing the burden on farmers and irrigation managers. As the field continues to evolve, addressing the challenges related to knowledge representation, validation, and integration will be crucial for successfully deploying and adopting of knowledge-based irrigation scheduling in sustainable agriculture.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

The authors declared that the following contributions are correct.

Abayomi AYODELE: Investigation, Methodology, Conceptualization, Data curation, Validation, Writing–original draft, Review, and Editing, Visualization.
Olugbenga OGIDAN: Investigation, Methodology, Conceptualization, Data curation, Validation, Writing–original draft, Review, and Editing, Visualization.
Adeseko AYENI: Investigation, Methodology, Data curation, Validation, Writing –original draft, Review, and Editing, Visualization.

ETHICS COMMITTEE DECISION

This article does not require any Ethical Committee Decision.

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A Critical Review on 'Current Status and Future Implications of Advanced Phenotyping Systems for Monitoring of Agricultural Crops'

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ABSTRACT

Phenotyping systems propels the growth of modern agriculture, driving innovations in plant breeding, crop management, precise application of resources and smart agriculture. This review provides a comprehensive analysis of phenotyping systems, exploring their status, technological advancements, challenges and future directions. The evolution from traditional phenotyping to high-throughput phenotyping (HTP) systems with involvement of advanced imaging (visible, infrared, hyperspectral, and thermal), sensors (LIDAR and NIR), data analytics, drones and automated platforms have enabled rapid non-invasive collection of phenotypic information, significantly hastening breeding programs and improving stress tolerance studies. The integration of big data, artificial intelligence (AI) and machine learning (ML) has enhanced data management and interpretation, enabling the development of predictive models and real-time decision-making tools. Despite these advancements, several challenges persist. The technical issues such as data accuracy, resolution and consistency alongside economic concerns related to high cost of implementation, limits the widespread adoption of advanced phenotyping technologies, especially among smallholder farmers. Furthermore, the integration of these technologies with traditional farming practices and the handling of large datasets raises concerns about data privacy, ownership and interpretation. The impending growth of phenotyping lies in advancements such as the integration of AI and genomics, enabling more precise breeding through the linking of genetic information with phenotypic traits. Additionally, the development of low-cost systems is essential to democratize access to precision agriculture, particularly in developing regions. As phenotyping systems continue to advance, they will play a critical role in promoting sustainable agriculture, enhancing resource efficiency, ensuring food security and addressing global climate change.

Keywords: *Phenotyping data, Agriculture, Technologies, Artificial intelligence, Challenges*

INTRODUCTION

Phenotyping intends for comprehensive assessment of observable traits of a crop or organism, such as morphology, development, biochemical properties and behavior in response to environmental conditions (Li *et al.*, 2014). In the context of agriculture and plant sciences, phenotyping is critical for understanding and establishing the relationship between genotype and phenotype, which is fundamental for crop improvement and breeding programs (Furbank and Tester, 2011). The importance of phenotyping in agriculture lies in its ability to facilitate the selection of superior genotypes with desired traits in terms of drought tolerance, disease resistance and yield potential (Reynolds *et al.*, 2020). The measurement of the parameters can provide a basis for the breeders to make informed decisions, leading to the expansion of crops that are better adapted to changing environmental conditions and accelerating the prospectus of feeding 9 billion people in 2050 and 11 billion in 2100 (Araus and Cairns, 2014; Muzamil *et al.*, 2022). Over the years, phenotyping system has gained prominence owing to its association with precision and smart agriculture. The smart agricultural practices are governed by its ability to provide instantaneous and real time data on crop characteristics and performance, that has the potential to augment resource use and enhance productivity (Shakoor *et al.*, 2017). Historically, the emergence of phenotyping system was intended to understand the complex traits in plants growth system. The evolution has been marked by the transformation from manual, labor-intensive and drudgery laced methods to highly automated sensor-based throughput systems, Figure 1.

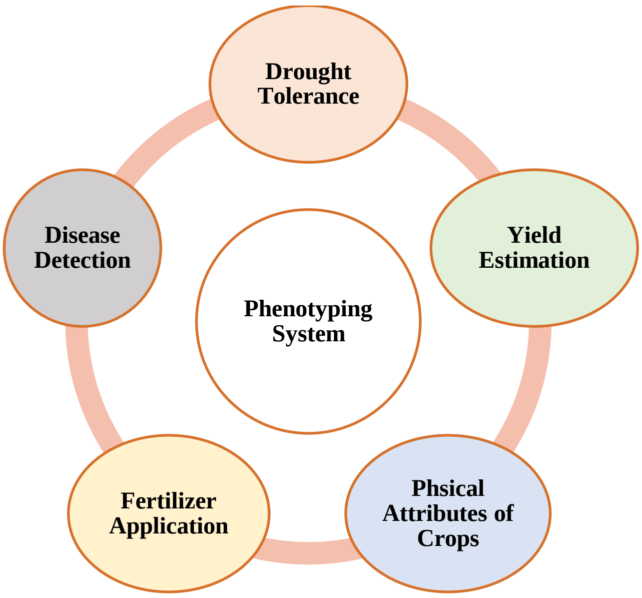

Figure1. *Applications of phenotyping system in agriculture.*

Initially, phenotyping in agriculture was predominantly conducted through manual observations and dimensions of plant traits such as height, leaf size and yield, which were time-consuming and prone to human errors (Xie *et al.*, 2021). The advent of imaging technologies in the late 20th century brought significant advancements, enabling more accurate and objective phenotypic assessments. The arrival and adoption of technologies like such as visible and near-infrared (NIR) imaging promoted non-invasive measurement of traits, providing more consistent and reproducible data (Li *et al.,* 2021). The application of phenotypic technologies in agriculture is enlisted in Table 1.

Table 1. *Application of phenotyping technologies in agriculture.*

Application	Technology	Refrences
1. Detection of disease symptoms in potato plants	Automated machine learning algorithms based high throughput phenotyping (HTP) system	Afzaal *et al.,* 2021
2. Phenotyping canola for plant traits	Automated phenomobile platform equipped with RGB and LiDAR sensors	Cao, 2018
3. Phenotyping wheat for nitrogen use efficiency (NUE)	Multispectral imaging system to measure canopy reflectance and chlorophyll content	Yang *et al.,* 2020
4. Measuring traits in large-scale rice trials	Drone-based HTP system	Panday *et al.,* 2020
5. Screening barley for heat tolerance	Infrared thermography system to capture temperature data from canopies	Kim *et al.,* 2018
6. Root phenotyping for Arabidopsis	Automated root phenotyping system using time-lapse imaging	Satbhai *et al.,* 2017
7. Estimating maize yield potential	LiDAR-based high-throughput phenotyping (HTP) system	Luo *et al.,* 2021
8. Yield and leaf area index estimation for groundnut crop	UAV-based high-throughput phenotyping system with multispectral cameras	Tahir *et al.,* 2020
9. Assessing water-use efficiency in cotton	UAV-based high-throughput phenotyping system for measurement of temperature and spectral reflectance	Lacerda *et al.,* 2022
10.Screening barley for fungal disease resistance	Hyperspectral imaging system	Zhou *et al.,* 2019
11. Screening wheat and rice for drought tolerance	UAV-based systems equipped with thermal and multispectral sensors	Chaturvedi *et al.,* 2019
12. Early-stage detection of stress in maize	Hyperspectral imaging system in high-throughput platform	Asaari *et al.,* 2019
13. Root architecture phenotyping for soybean	X-ray CT imaging to generate 3D root images	Nakhforoosh *et al.,* 2024
14. Evaluating crop phenology in coffee plants	UAV-based phenotyping with RGB and multispectral cameras	Barbosa *et al.,* 2021
15. Phenotyping for drought tolerance in sorghum	UAV-based remote sensing system	Li *et al.,* 2018
16. Estimating yield in rice	Hyperspectral imaging to predict yield from spectral data	Kurihara *et al.,* 2023
17. Monitoring fruit size and color in tomato plants	RGB and hyperspectral imaging in automated greenhouse systems	Deulkar and Barve, 2018
18. Measuring water-use efficiency in wheat	UAV-based thermal and multispectral imaging systems	Bhandari *et al.,* 2021

In recent years, phenotyping systems have further evolved with the integration of high-throughput platforms, which can process and analyze large datasets in a short span of time. These systems utilize advanced sensors, robotics and data analytics to capture, record and analyze phenotypic data at an unprecedented scale (Atefi *et al.,* 2021). The use of drones, UGV (unmanned ground vehicles) and UAV (unmanned aerial vehicles) in field phenotyping has also revolutionized the ability to monitor crops over large areas, providing insights into spatial variability, temporal inconsistencies and environmental interactions (Tanaka *et al.,* 2024). The incorporation of artificial intelligence (AI) and machine learning (ML) in phenotyping systems has heightened the ability to analyze multifaceted datasets, leading to more precise estimates and predictions of plant performance under various conditions (Nabwire *et al.,* 2021). This technological evolution continues to push the boundaries of phenotyping, enabling more efficient breeding programs and precision agriculture practices. The review paper highlights the major advancements in phenotyping systems from last decade with the help of published data. The literature was selected on the basis of availability, relevance, economic viability, technical superiority and feasibility to be deployed at actual fields.

EVOLUTION OF PHENOTYPING SYSTEMS

The phenotyping technologies of the crop system depends on the technological interventions and situations. Initially, there were only two classifications-manual phenotyping and high throughput phenotyping system, Figure 2. However, it has expanded to different sectors of agricultural sections including green house, UAV and precision agriculture, Table 2.

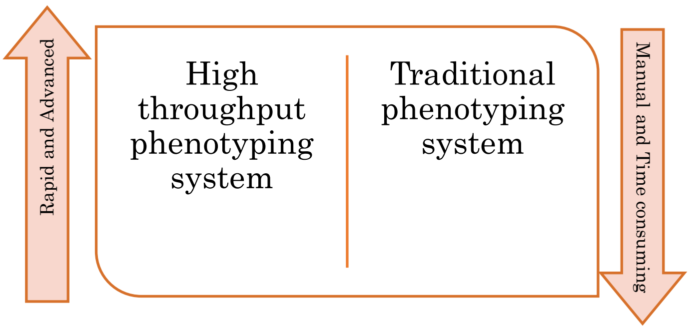

Figure 2. *Types of phenotyping system in agricultural system.*

Table 2. Different types of phenotyping technologies employed in agriculture.

Type	Description	Reference
Traditional Phenotyping	Manual measurement and visual assessment of plant traits without the use of advanced technologies	Maqbool *et al.*, 2022
High-Throughput Phenotyping (HTP)	Automated systems that use sensors, imaging, and computational tools to rapidly measure plant traits in large-scale studies.	Asaari *et al.*, 2019; Luo *et al.*, 2021
Field-based Phenotyping	HTP conducted in real agricultural fields using drones or mobile platforms.	Chaturvedi *et al.*, 2019; Tahir *et al.*, 2020
Greenhouse-based Phenotyping	Automated platforms in controlled environments like greenhouses that monitor plant traits using imaging and sensors.	Deulkar and Barve, 2018
Root Phenotyping	Specialized techniques for assessing below-ground traits like root architecture and water/nutrient uptake.	Nakhforoosh *et al.*, 2024
UAV-based Phenotyping	Unmanned Aerial Vehicles (drones) equipped with sensors to capture phenotypic data from large agricultural areas.	Bhandari *et al.*, 2021; Asaari *et al.*, 2019
Imaging-based Phenotyping	Use of various imaging techniques (visible, thermal, hyperspectral, multispectral) to assess plant health and physiological traits.	Kurihara *et al.*, 2023
Root and Canopy Phenotyping	Combined systems that measure both above-ground and below-ground plant traits to assess overall plant health and productivity.	Luo *et al.*, 2021
Precision Agriculture Systems	Phenotyping integrated with precision agriculture technologies for real-time decision-making in farm management.	Araus *et al.*, 2022

Traditional Phenotyping

Traditional phenotyping relies on utilizing the manual measurements and visual assessments for plant characterization. This method is based on the skill of the worker to understand the situation and measure or record the parameters accordingly. This method is characterized by cost-effectiveness, simplicity and the ability to capture complex traits that automated systems may overlook. They provide detailed and context-specific data, essential for understanding complex plant traits and interactions. Although high-throughput technologies are rapidly advancing, traditional methods will continue to play a vital role in plant research and breeding, particularly in validating new technologies, conducting detailed trait analyses and supporting agricultural development in resource-limited settings (Brown and Miller, 2019; Zhang *et al.*, 2021). However, traditional phenotyping is valuable, particularly in resource-limited settings, for assessing traits that are difficult to quantify with technology and validating data obtained from high-throughput systems (Dogan *et al.*, 2018).

Despite the advent of advanced phenotyping systems, traditional methods remain indispensable in many agricultural and plant science contexts, Table 1. Traditional phenotyping methods have been the backbone of plant science for decades, providing fundamental insights into plant progress, growth, progress and responses to environmental strains. These methods are highly valuable in regions with limited access to advanced technologies and resources, enabling researchers and farmers to assess crop performance effectively (Singh *et al.,* 2021). Traditional approaches are essential for validating and calibrating data obtained from modern phenotyping platforms, ensuring accuracy and reliability in trait measurements (Reynolds *et al.,* 2020). Traditional phenotyping is also preferred when dealing with complex traits that require detailed and nuanced assessments, which may not be fully captured by automated systems. The traits such as leaf texture, disease symptoms and specific developmental stages often necessitate expert visual evaluation to ensure precise characterization (Lee *et al.,* 2020).

Traditional phenotyping to assess drought tolerance in maize, measuring traits like leaf wilting and chlorosis (Fisher *et al.,* 2015) found that these methods provided reliable data crucial for selecting drought-tolerant varieties for smallholder farmers, Figure 3. Traditional phenotyping was to evaluate wheat cultivars for leaf rust resistance, employing detailed visual inspections and standardized scoring scales. This method enabled precise identification of resistant genotypes and supported effective breeding strategies, as the complexity of disease symptoms required expert interpretation beyond current imaging technologies. Sinesio *et al.* (2021) assessed fruit quality traits like flavor, texture and aroma in various tomato varieties using traditional sensory evaluation with human panels emphasizing that human sensory analysis is important for capturing the subjective and complex aspects of fruit quality that automated systems struggle to quantify. Maqbool *et al.* (2022) studied root architecture in rice by using traditional excavation and manual measurement techniques. Despite its labor-intensive nature, this approach offered detailed and accurate data on root length, density, and branching patterns, which is crucial for breeding programs focused on improving nutrient and water uptake efficiency. Traditional phenotyping was employed to measure plant height, leaf area and biomass in wild populations aiming to understand adaptation to various ecological niches, highlighting that traditional methods provide the flexibility and adaptability needed for field studies in diverse and challenging environments (Diaz-Garcia *et al.* 2024). Traditional observational techniques to track soybean growth stages (Gupta *et al.,* 2020) across various climatic zones showed that manual observations delivered timely and accurate data, which was essential for effectively scheduling irrigation, fertilization and pest control. Manual measurement techniques to evaluate the impact of salinity stress on barley seedlings in controlled environments (Nguyen *et al.,* 2019) enabled detailed analysis of physiological responses under controlled conditions. Enhancing the nutritional quality of crops is a key objective in breeding programs. Traditional laboratory analyses to measure protein, mineral and vitamin content yielded accurate and reliable data imperative for breeding nutritionally enhanced crop varieties.

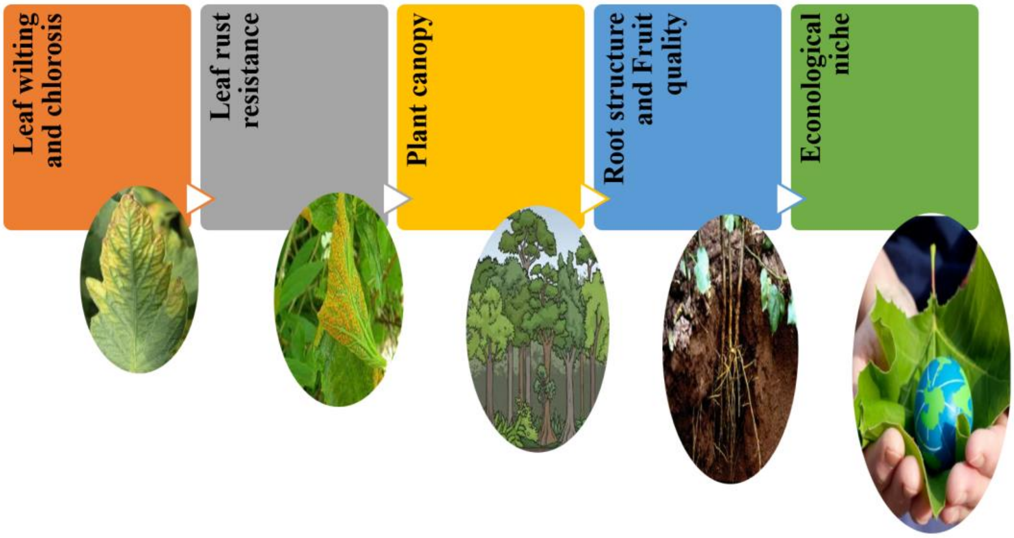

Figure 3. *Phenotyping system for measurement of plant characteristics.*

Traditional phenotyping plays a crucial role in the characterization and conservation of plant genetic resources. Alonso *et al.* (2020) performed manual assessments of morphological and agronomic traits in heirloom vegetable varieties to document and preserve their unique characteristics. The study underscored the importance of traditional methods in maintaining biodiversity and supporting sustainable agriculture. In participatory breeding programs involving farmers and local communities, traditional phenotyping methods are integral. Rodriguez *et al.* (2019) collaborated with farmers to evaluate and select maize varieties based on manual assessments of yield, taste and adaptability. This approach empowered local stakeholders and ensured that selected varieties met the specific needs and preferences of end-users. Müller and Becker (2020) utilized traditional field measurements to assess heat and drought tolerance in sorghum. Parameters such as plant height, leaf area and grain yield were manually recorded under varying stress conditions. The study demonstrated that traditional phenotyping provides robust data critical for developing stress-resilient crop varieties. Understanding interactions between plants and soil microbes often requires traditional assessment methods. Li *et al.* (2021) conducted manual measurements of root exudates and soil nutrient levels to study mutualistic relationships influencing plant health and productivity. The detailed analyses facilitated insights that are challenging to capture through automated systems. In the tea industry, traditional sensory evaluation remains the standard for assessing quality and flavor profiles. Kim and Lee (2019) employed expert tasters to evaluate different tea cultivars, providing nuanced assessments essential for maintaining product standards and guiding breeding efforts aimed at flavor improvement.

Research on phenotypic plasticity often relies on traditional phenotyping to capture variability in plant responses to change in environmental parameters. Fernandez *et al.* (2020) used manual measurements to study how different light conditions affected leaf morphology and photosynthetic rates in forest understory species. Detecting and managing herbicide resistance in weeds is important for crop protection. Thompson and Carter (2019) performed traditional bioassays involving manual observation and measurement of weed growth following herbicide application. This method provided direct and reliable assessments necessary for effective resistance management strategies. In the aftermath of natural disasters, traditional phenotyping methods are often employed to quickly assess crop damage and plan recovery efforts. Oliveira *et al.* (2021) conducted field surveys using manual observations to evaluate the impact of flooding on rice fields, facilitating timely and informed decision-making for restoration. Urban agriculture projects frequently utilize traditional phenotyping due to space and resource constraints. Williams *et al.* (2019) incorporated manual measurement exercises in their curriculum to teach fundamental concepts of plant morphology and physiology, emphasizing hands-on learning and skill development. Traditional methods are important for validating and calibrating data obtained from high-throughput phenotyping systems. Walter *et al.* (2019) conducted parallel manual and automated measurements of wheat canopy traits to ensure the accuracy and reliability of HTP data, highlighting the complementary role of traditional approaches. In many developing countries, traditional phenotyping remains the primary method due to limited access to advanced technologies. Ahmed *et al.* (2021) concluded that extensive manual evaluations of millet varieties under local field conditions contributes valuable data for improving food security and agricultural resilience in resource-constrained regions.

High-throughput Phenotyping (HTP): High-throughput phenotyping (HTP) systems use advanced technologies such as imaging sensors, robotics, and computational tools for rapid and non-invasive measurement of plant traits in large-scale breeding programs. The advent of HTP has also accelerated the process of developing climate-resilient crops that can withstand environmental stresses like drought, heat, and salinity (Nabwire *et al.,* 2021). These systems are designed to handle large volumes of plants while capturing a wide range of phenotypic traits across diverse environments and time points. HTP has revolutionized modern agriculture by enabling the efficient selection of genotypes with superior traits for higher productivity. HTP has improved crop breeding programs by integrating sensors such as RGB cameras, thermal imaging, LiDAR, and hyperspectral imaging to increase the speed and accuracy of phenotypic data collection in terms of plant structure, health, and physiological responses (Mahlein *et al.,* 2018). The ability of HTP to operate in controlled environments like greenhouses, as well as in open fields, makes it versatile for evaluating crops under real-world agricultural conditions, Figure 4. HTP has facilitated the study of complex traits such as water-use efficiency, photosynthetic capacity and root architecture, which are challenging to measure manually.

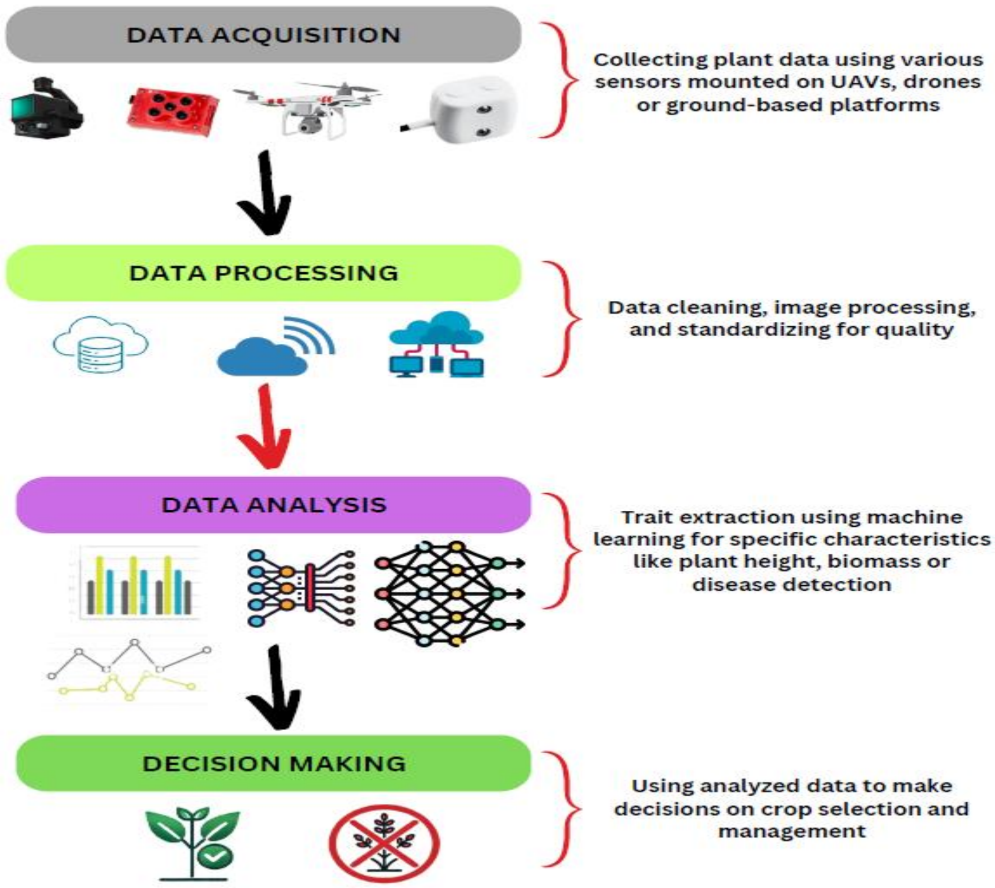

Figure 4. *Process methodology of HTP system.*

Bhandari *et al.* (2021) used UAVs with thermal and multispectral cameras to phenotype wheat genotypes under drought conditions. The UAVs collected canopy temperature and NDVI data, aiding in the identification of drought-tolerant lines. This high-throughput phenotyping (HTP) approach enhanced the accuracy of trait measurement and significantly reduced the time needed for phenotyping. Kurihara *et al.* (2023) utilized hyperspectral imaging to predict rice yield in large field trials by analyzing reflectance data from various spectral bands. This method enabled highly accurate yield estimation before harvest, facilitating earlier selection of high-yielding varieties and shortening the breeding cycle. Deulkar and Barve (2018) used an automated phenotyping platform in a greenhouse study to measure fruit size, shape, and color in tomato plants. By utilizing RGB and hyperspectral imaging, the system continuously monitored the ripening process and detected defects in fruit quality. This high-throughput phenotyping (HTP) system enabled the selection of tomato varieties with superior fruit quality. Asaari *et al.* (2019) employed field-based high-throughput phenotyping (HTP) to assess drought tolerance in maize hybrids. Using a ground-based system with thermal and LiDAR sensors, they collected data on canopy temperature, plant height, and biomass. The study identified drought-tolerant maize hybrids, which were later integrated into breeding programs. Li *et al.* (2018) focused on sorghum, a key crop for food and bioenergy, using an HTP platform with drones and ground-based sensors to measure biomass traits like plant height, leaf area, and chlorophyll content. This system enabled rapid screening of sorghum genotypes, leading to the identification of high-biomass-producing lines.

Nakhforoosh *et al.* (2024) used X-ray CT imaging to phenotype root architecture in soybean, generating detailed 3D images of root structures. This non-destructive method allowed the identification of genotypes with more efficient root systems for water and nutrient uptake, surpassing the limitations of traditional phenotyping techniques. An automated high-throughput phenotyping (HTP) system that used machine learning algorithms (Afzaal *et al.,* 2021) was developed to detect disease symptoms in potato plants. By capturing high-resolution images at various growth stages, the system employed artificial intelligence to identify early signs of disease, significantly reducing the time and labor involved in monitoring large potato fields. An automated phenomobile platform equipped with RGB and LiDAR sensors (Cao, 2018) was used to phenotype canola plants, measuring traits like plant height, leaf area index and flowering time. The platform efficiently covered large field plots, offering high-throughput data to support canola breeding programs. Yang *et al.* (2020) employed multispectral imaging to phenotype wheat plants for nitrogen use efficiency (NUE). The high-throughput system measured canopy reflectance and chlorophyll content, which were linked to NUE. The study successfully identified wheat genotypes with enhanced nitrogen uptake, aiding in the development of more sustainable cropping systems.

Drone-based high-throughput phenotyping (HTP) measured traits like plant height, biomass and leaf area in a large-scale rice cultivation (Panday *et al.,* 2020). The drones enabled rapid data collection across multiple field sites, accelerating the breeding process and facilitating the selection of high-performing rice varieties. Infrared thermography to phenotype heat tolerance in barley (Kim *et al.* 2018) captured temperature data from barley canopies, allowing in identifying genotypes that maintained lower canopy temperatures under heat stress. Satbhai *et al.* (2017) developed an automated root phenotyping system for Arabidopsis using time-lapse imaging to monitor root growth and development. The system captured high-resolution data on root length, branching and angle, allowing for the rapid screening of Arabidopsis mutants with modified root architectures. A Phenotyping tool (RhizOSun) with Raspberry Pi computer and a picamera for acquiring images was employed for automatic recording of the number of tubercles counted on sunflower root (Le Ru *et al.,* 2021).

Luo *et al.* (2021) used LiDAR-based high-throughput phenotyping (HTP) to estimate yield potential in maize. The LiDAR system scans maize fields to create 3D models of plant structures, which are then used to estimate biomass and grain yield. The study showed that LiDAR effectively provided accurate yield predictions for maize breeding programs. The UAV-based high- phenotyping system with multispectral cameras was used to estimate real time leaf area index and yield of groundnut crop utilizing Normalized Difference Vegetation Index (NDVI) (Tahir *et al.,* 2020). The system allowed for rapid phenotyping of large breeding plots, leading to the identification of genotypes with improved yield and disease resistance. A UAV-based HTP system (Lacerda *et al.,* 2022) to assess water-use efficiency in cotton was employed to measure canopy temperature and spectral reflectance which were correlated with water-use efficiency. The HTP approach allowed for the identification of cotton lines with improved drought tolerance and water-use efficiency. An HTP platform (Zhou *et al.,* 2019) to screen barley lines for resistance to fungal diseases employed hyperspectral imaging to detect early symptoms of disease, enabling the identification of resistant genotypes invisible to naked eye. According to Barbosa *et al.* (2021), UAV integrated with RGB camera aligned with computer vision can help to measure coffee tree height/diameter and predict yield of coffee. The system used UAVs equipped with multispectral cameras to collect phenotypic data across a large coffee plantation, supporting breeding efforts for high-yielding and disease-resistant coffee varieties. Chaturvedi *et al.* (2019) utilized high-throughput phenotyping (HTP) to assess rice lines for drought and heat tolerance. UAVs equipped with thermal and multispectral sensors collected data on canopy temperature and NDVI, correlating these with drought and heat tolerance traits. The HTP system facilitated the identification of resilient rice varieties. Some of the applications of phenotyping technology in agriculture are highlighted in Table 2.

TECHNOLOGIES USED IN PHENOTYPING SYSTEM

Sensor Technologies: The use of sensor technologies in HTP systems has revolutionized the ability to collect detailed, real-time data on plant growth, development, and environmental responses. These sensors allow for the non-invasive assessment of a wide range of physiological and structural traits, significantly enhancing the precision of phenotyping. The integration of diverse sensor modalities, including RGB cameras, multispectral and hyperspectral imaging and LIDAR, has significantly improved the precision and efficiency of phenotyping efforts, Figure 5. One of the primary advantages of HTP systems is their ability to automate data collection, which reduces labor costs and human error. Ground-based robots equipped with imaging and LIDAR sensors can accurately measure plant height and biomass at high resolutions, allowing for detailed assessments of crop performance over time (Young *et al.,* 2019; Yao *et al.,* 2021). This automation is crucial for large-scale studies, where traditional manual measurements would be impractical. Moreover, the combination of various sensor types enhances the richness of the data collected, as different sensors can capture complementary information about plant health and growth dynamics (Ma *et al.,* 2022).

Figure 5. *Different sensors used in phenotyping system for agricultural crops.*

The recent advancements in sensor technologies have also facilitated the development of sophisticated technologies and data processing algorithms that can analyze the huge amounts of data generated by HTP systems (Deery *et al.,* 2014), Figure 5. Machine learning techniques have been employed to extract meaningful insights from phenotypic data, enabling researchers to identify genetic traits associated with desirable agronomic characteristics (Tsaftaris *et al.,* 2016; Yang *et al.,* 2020). Studies have demonstrated that integrating genomic data with phenotypic measurements can accelerate the identification of quantitative trait loci (QTLs) linked to yield components in crops like rice (Tanger *et al.,* 2017; Wu *et al.,* 2019). The various types of sensors used in phenotyping system are shown in Table 3.

Table 3. *Different types of sensors used in phenotyping systems.*

Type of Sensor	Description	References
RGB Cameras	Capture visible light in red, green, and blue bands, commonly used for basic morphological traits like plant height, leaf area, and fruit size.	Deulkar and Barve, 2018; Zhang *et al.,* 2023
Thermal Infrared Sensors	Measure plant surface temperature to assess water status and heat stress tolerance by detecting canopy temperature.	Banerjee *et al.,* 2020; Lacerda *et al.,* 2022
Multispectral Sensors	Capture reflectance data across several bands (e.g., NIR, red, and blue) to calculate vegetation indices like NDVI.	Tahir *et al.,* 2020; Panday *et al.,* 2020
Hyperspectral Sensors	Capture data across hundreds of spectral bands to assess physiological traits, such as chlorophyll content and nitrogen status	Asaari *et al.,* 2019; Kurihara *et al.,* 2023
LiDAR (Light Detection and Ranging)	Use laser beams to generate 3D models of plant structures, accurately measuring height, biomass, and canopy traits.	Luo *et al.,* 2021; Yao *et al.,* 2021
X-ray CT (Computed Tomography)	Non-invasive imaging to generate 3D root system models, enabling accurate root phenotyping.	Nakhforoosh *et al.,* 2024
Near-Infrared (NIR) Sensors	Capture near-infrared light, typically used for monitoring water status and photosynthetic activity.	Yang *et al.,* 2020; Araus *et al.,* 2022
Spectral Reflectance Sensors	Measure how much light is reflected by plants, used to evaluate health, nutrient content, and stress levels.	Yang *et al.,* 2020
Fluorescence Sensors	Measure chlorophyll fluorescence to assess photosynthetic efficiency and plant stress responses.	Mahlein *et al.,* 2018
Ultrasound Sensors	Measure root traits such as diameter and length, providing non-invasive phenotyping of roots.	Nguyen *et al.,* 2019

This integration is essential for breeding programs focusing to enhance crop resilience and efficiency in the face of climate change. The application of remote sensing technologies has revolutionized the way phenotyping is conducted. Aerial platforms, such as drones, equipped with multispectral cameras, allow for the monitoring of large fields and the assessment of crop conditions over extensive areas (Thorp *et al.,* 2018; Araus *et al.,* 2022), Figure 6. These technologies not only provide spatially explicit data but also enable real-time monitoring of plant responses to environmental stresses, such as drought or nutrient deficiency. The ability to capture dynamic changes in plant phenotypes is critical for developing strategies to improve water use efficiency and overall crop performance (Thorp *et al.,* 2018; Yuan *et al.,* 2023).

Components
1. Frame
2. Wheel encoders
3. GPS
4. Adjustable height boom
5. Light bank
6. LiDAR sensor
7. RGB stereo camera
8. Spectrometer/hyperspectral camera
9. IR thermometer
10. Generator and electronics
11. Hydraulic drive

Figure 6. *Advanced HTP system with sensor integration (Deery et al., 2014).*

Despite the advancements in sensor technologies, challenges remain in the standardization and integration of data across different platforms. Variability in sensor calibration, environmental conditions, and data processing methodologies can introduce biases that complicate data interpretation (Wang *et al.,* 2018; Roitsch *et al.,* 2019). Therefore, ongoing research is focused on developing standardized protocols and robust data management systems to ensure the reliability and comparability of phenotypic data across studies (Zhao *et al.,* 2019; Ma *et al.,* 2022). Sensor technologies are at the forefront of high-throughput phenotyping systems, providing unprecedented opportunities for crop research and breeding. The integration of diverse sensor modalities, coupled with advanced data analytics, is transforming the landscape of agricultural science. As these technologies continue to grow, they hold the potential to significantly enhance our understanding of plant biology and improve agricultural productivity in a sustainable manner.

Imaging Techniques: Imaging techniques have become fundamental to high-throughput phenotyping (HTP) systems, enabling non-invasive and high-precision data collection on a range of plant traits. These techniques employ different parts of the electromagnetic spectrum to gather information on plant health, growth, stress responses and other important characteristics. The visible imaging, which captures information within the range of the human eye (400-700 nm), is one of the simplest and most cost-effective methods in phenotyping. It provides high-resolution images of plant architecture, including traits like plant height, leaf area, and color (Shakoor *et al.,* 2017). RGB cameras are commonly used to assess morphological traits such as leaf angle and fruit size in crops like maize and tomato (Zhang *et al.,* 2023). Despite its simplicity, visible imaging can be limited in detecting physiological changes, particularly in early stages of stress or disease (Deulkar and Barve, 2018). Infrared (IR) imaging, particularly in the thermal infrared range (8-14 μm), is used to assess plant temperature, which is a proxy for water status and heat stress tolerance. IR imaging systems measure the radiation emitted by plants, enabling the detection of transpiration rates and plant water use efficiency (He *et al.,* 2024). IR imaging has been used to screen for drought-tolerant genotypes by identifying plants that maintain cooler canopy temperatures under water deficit conditions (Banerjee *et al.,* 2020). Hyperspectral imaging captures information from a wide range of wavelengths (typically 400-2500 nm) and is particularly useful for assessing plant physiological traits such as chlorophyll content, nutrient status and disease severity (Sarić *et al.,* 2022).

Hyperspectral cameras divide the light spectrum into hundreds of narrow bands, allowing the detection of subtle differences in plant reflectance that may not be visible to the human eye. Hyperspectral imaging has been successfully used in rice to predict yield and detect nitrogen deficiencies (Kurihara *et al.,* 2023). Multispectral imaging operates in fewer wavelength bands than hyperspectral imaging (typically 3-12 bands), but it still provides valuable insights into plant health. Multispectral sensors measure plant reflectance at key wavelengths, such as near-infrared (NIR), red and blue, which are commonly used to calculate vegetation indices like the NDVI (Normalized Difference Vegetation Index) (Roberts *et al.,* 2018). These indices are highly correlated with photosynthetic activity, biomass, and plant vigor. Multispectral imaging is frequently employed in field-based phenotyping using drones, especially for crops like maize and wheat (Zaman-Allah *et al.,* 2015). Thermal imaging, a type of infrared imaging, focuses specifically on capturing the temperature of plant surfaces. It plays a critical role in monitoring plant responses to heat stress and water availability (Zhu *et al.,* 2018). By measuring canopy temperature, thermal imaging can help breeders select heat-tolerant and drought-resistant crops.

Data Management and Analysis: Effective data management and analysis are critical in modern phenotyping systems, especially in high-throughput phenotyping (HTP), where large volumes of complex data are generated. Technologies like big data, artificial intelligence (AI) and machine learning (ML) are increasingly applied to handle and interpret this data, leading to improved breeding decisions and more efficient crop management. The rise of HTP platforms has resulted in an explosion of data from various sources, including imaging, sensors, environmental monitoring, and genomic information (Fiorani and Schurr, 2019). Managing this data requires advanced big data technologies that can handle the integration of diverse datasets. These technologies allow for the analysis of large-scale phenotypic, environmental, and genetic data, enabling more comprehensive breeding decisions. The use of big data technologies to manage multi-location trials (Tardieu *et al.,* 2017) allows researchers to integrate phenotypic data across varied environmental conditions to identify genotypes with stable performance, helping in understand genotype-environment interactions and make more targeted selections in breeding programs. The ability to manage large, diverse datasets is essential in identifying the best-performing crops under different stress conditions (Krause *et al.,* 2019). Artificial Intelligence is revolutionizing phenotyping by automating the interpretation of large datasets. AI algorithms, particularly those based on deep learning, have proven highly effective in analyzing image and sensor data (Singh *et al.,* 2016). Deep learning models can identify subtle patterns in plant images, such as leaf texture or color, which are often early indicators of diseases (Pound *et al.,* 2017). Deep learning models have been used to detect leaf blight in rice by analyzing digital images and comparing them to historical data (Kamilaris and Prenafeta-Boldú, 2018). AI-based systems have been applied to predict crop yields by correlating multispectral images with historical yield data. A study on maize demonstrated that AI models could predict final crop yields based on image data collected during early growth stages, offering real-time insights into crop health and performance (Yang *et al.,* 2022). Such predictive tools are invaluable in improving resource allocation and decision-making for farmers and breeders alike.

Machine learning (ML) methods are essential for processing high-dimensional phenotypic data and identifying non-linear relationships between traits and environmental factors. In crop phenotyping, ML models can predict crop performance based on phenotypic data collected under varying environmental conditions (Araus *et al.,* 2012). ML models have been used to analyze phenotypic traits like canopy temperature and chlorophyll content to predict drought tolerance in maize (Montesinos-López *et al.,* 2021). Machine Learning has also facilitated genotype-phenotype association studies by analyzing large-scale phenotypic data alongside genomic information allowing researchers to identify genes linked to desirable traits such as disease resistance or yield potential (Crossa *et al.,* 2017). ML techniques help in identifying genomic regions associated with high yield, accelerating breeding cycles by enabling breeders to focus on high-potential genotypes early in the process (Li *et al.,* 2024).

CHALLENGES AND LIMITATIONS OF CURRENT PHENOTYPING SYSTEMS

Technical Challenges: High-throughput phenotyping (HTP) systems have brought immense potential for improving agricultural practices and breeding programs. However, they face several technical challenges, particularly related to data accuracy, resolution, and consistency. Addressing these challenges is essential for maximizing the potential of phenotyping technologies. One of the major challenges in phenotyping systems is ensuring data accuracy. Phenotyping platforms rely heavily on sensors, imaging systems, and automated data collection processes, which can introduce errors due to sensor limitations, calibration issues and environmental noise. Inaccurate sensor calibration or poor lighting conditions can result in incorrect measurements of plant height or leaf area, affecting the reliability of the data (Fiorani and Schurr, 2019).

In field-based phenotyping systems, environmental variability further complicates data accuracy. Factors such as wind, rain, and soil heterogeneity can impact the precision of measurements, especially when drones or mobile platforms are used for data collection (Wang *et al.,* 2024). Additionally, plant movements caused by wind can skew measurements in real-time, leading to inaccurate assessments of canopy structure (Feng *et al.,* 2021). Another critical technical limitation is the resolution of data, particularly in imaging-based phenotyping systems. While high-resolution imaging technologies such as hyperspectral or multispectral cameras can capture fine details of plant traits, there is often a trade-off between resolution and the speed of data acquisition. High-resolution imaging systems may slow down the data collection process or increase computational demand for processing, making it difficult to apply these systems in large-scale field trials (Shi *et al.,* 2021). In remote sensing applications, resolution is also limited by the altitude at which drones or satellites operate. Higher altitudes reduce spatial resolution, potentially missing subtle phenotypic traits such as leaf disease spots or early signs of water stress (Xue and Su, 2017). Low-resolution data can also mask small differences between genotypes, making it harder to distinguish superior-performing varieties during selection (Walter *et al.,* 2019).

Ensuring data consistency is another significant challenge in phenotyping. Consistency is affected by the variability of environmental conditions, measurement timing, and differences in phenotyping protocols. The same crop trait measured under different lighting conditions or at different times of day can yield varying results (Gill *et al.,* 2022). Moreover, inconsistency in sensor performance, caused by sensor drift or changes in calibration over time, can reduce the reliability of long-term studies (Ge *et al.,* 2016). Phenotyping systems that are used across multiple locations or seasons face additional consistency challenges. Variations in weather, soil type, and agricultural practices can lead to inconsistent data, making it difficult to compare results across different environments (Araus *et al.,* 2022). Standardizing data collection methods and ensuring uniformity in phenotyping protocols are crucial for improving consistency in multi-location trials.

Economic Considerations: The adoption of advanced phenotyping systems, particularly high-throughput phenotyping (HTP), presents significant economic challenges. The costs associated with implementing and maintaining these systems can be substantial, particularly for small-scale farmers or research institutions with limited budgets. The upfront investment required to establish a phenotyping system, especially HTP platforms, can be prohibitively expensive. The costs include purchasing advanced imaging equipment, sensors, automated platforms, and the necessary computational infrastructure for data storage and analysis (Fiorani and Schurr, 2019). Hyperspectral imaging systems and LiDAR sensors, which are commonly used in phenotyping can cost tens of thousands of dollars (Wang *et al.,* 2024). Additionally, the need for high-powered computing resources to process large datasets further increases the initial cost of implementation (Zhao *et al.,* 2019). Custom-built phenotyping platforms such as phenomobiles (mobile platforms equipped with sensors) or drones require not only specialized equipment but also technical expertise for their operation and maintenance (Shi *et al.,* 2023). Moreover, integrating these platforms with data management systems and ensuring that they are compatible with existing agricultural practices adds to the complexity and cost of implementation (Rico-Chávez *et al.,* 2022).

Jimenez-Berni *et al.* (2018) attempted to design and develop a low cost phenomobile system with sensor attachments for monitoring of crops on real time basis, Figure 7.

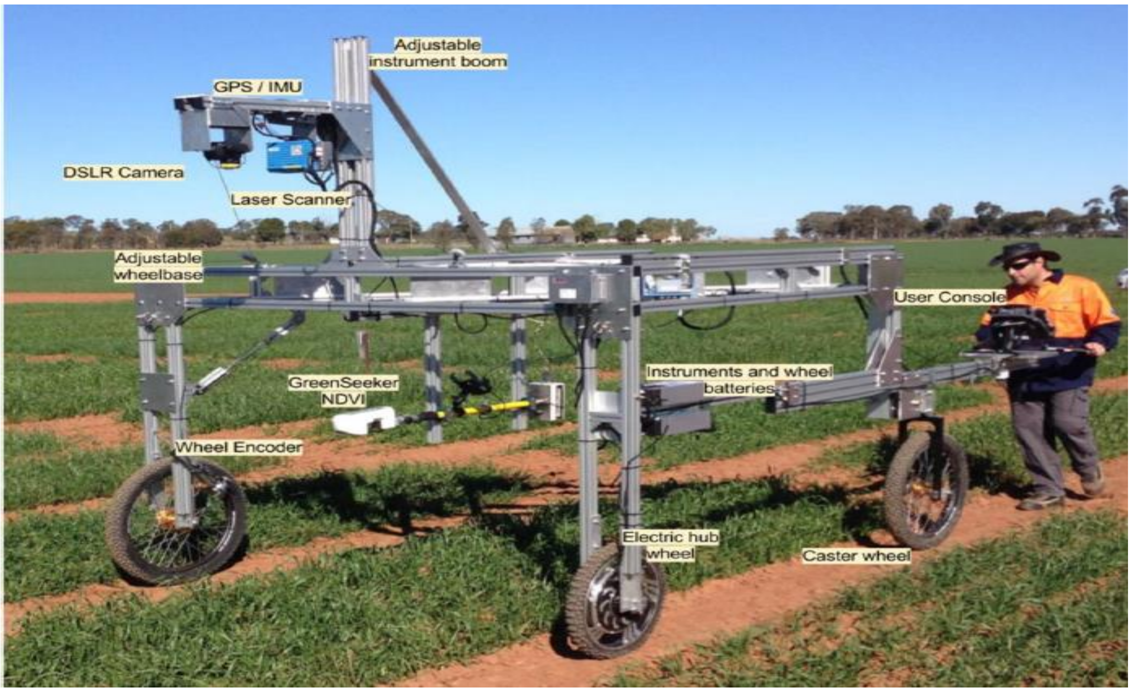

***Figure** 7. Low cost phenomobile platform and sensor attachments (Jimenez Berni et al., 2018).*

The maintenance of phenotyping systems is another important economic factor. Advanced phenotyping platforms, such as drones, robotic systems and automated imaging equipment, require regular calibration and servicing to maintain data accuracy (Kamarianakis *et al.,* 2024). Sensor performance can degrade over time, necessitating frequent recalibration or replacement. The cost of maintaining these systems is compounded by the need for skilled technicians to operate and troubleshoot them (Mao *et al.,* 2017). In field-based systems, environmental factors such as dust, humidity, and extreme weather conditions can affect the durability and performance of equipment, leading to higher maintenance costs. Drones used in field phenotyping may require frequent repairs or part replacements due to exposure to harsh outdoor conditions (Washburn *et al.,* 2024). Similarly, automated greenhouse systems, which involve moving platforms and robotic arms need regular upkeep to ensure smooth operation.

The operation of HTP systems often requires highly trained personnel to manage both the hardware and software components. Training staff to operate advanced imaging systems, interpret sensor data, and manage big data infrastructure incurs additional costs (Kim *et al.,* 2020). Even with automation, skilled labor is required to oversee data collection, analyze results, and troubleshoot technical issues (Araus *et al.,* 2022). Smaller institutions or research farms may not have the resources to hire specialized staff, making the operation of such systems more costly and less feasible. While HTP systems offer tremendous benefits for large-scale breeding programs and research, their high costs make them less accessible for smallholder farmers and low-resource institutions (Kaur *et al.,* 2024). Scaling down these systems to make them affordable for broader use remains a challenge. Small-scale implementations may still require significant investment, and while these costs may be justifiable for large research programs, they are often too high for smaller operations.

Despite the high costs of implementation and maintenance, the potential economic benefits of phenotyping systems cannot be ignored. By improving the efficiency of breeding programs, increasing crop yields, and reducing input costs, HTP platforms can lead to long-term economic gains (Costa *et al.,* 2019). However, realizing these benefits requires a significant upfront investment, which may be a barrier for widespread adoption, especially in developing countries or regions with limited agricultural funding (Feng *et al.,* 2021).

Integration with Existing Agricultural Practices: High-throughput phenotyping (HTP) systems are reshaping agricultural research and crop improvement, but integrating these technologies into traditional farming practices poses several challenges. Smallholder farmers continue to rely on manual labor, conventional tools, and historical knowledge, making the transition to advanced phenotyping more complex. Several factors must be addressed for successful integration. One significant barrier is the complexity of advanced phenotyping systems. HTP platforms involve sophisticated tools such as drones, multispectral cameras, and environmental sensors that require specialized knowledge to operate effectively. Traditional farmers familiar with visual assessments may struggle with systems that rely on machine learning algorithms for decision-making. Studies have highlighted the need for comprehensive training programs to ensure that farmers can adapt to these technologies and maximize their utility in field settings (Mir *et al.,* 2019; Ruzzante *et al.,* 2021). Infrastructure deficits are another roadblock. In many regions, particularly in developing countries, access to reliable electricity, internet connectivity, and data management systems remains inadequate (Singh, 2022). This lack of infrastructure impedes the adoption of HTP platforms, which rely on consistent data transmission and analysis. Furthermore, the high cost of equipment poses an economic barrier for smallholder farmers, making external support crucial for adoption (Hatem *et al.,* 2022).

The lack of standardization across phenotyping systems is a significant issue for integration. Current technologies vary widely in their data formats, making it difficult for farmers to incorporate these systems into their existing workflows. Moreover, traditional farming often involves multiple crop varieties and environmental conditions, which complicates the implementation of generalized phenotyping solutions (Wang *et al.,* 2024). Customization of phenotyping systems for specific crops or regions is essential for widespread adoption. There is a notable gap between the technical knowledge required for modern phenotyping systems and the traditional expertise of farmers. Traditional farmers are skilled at observing visual signs of plant health, but they may find sensor-generated data challenging to interpret (Jimenez-Berni *et al.,* 2018). Bridging this knowledge gap requires investment in educational initiatives to help farmers understand the benefits and practical applications of phenotyping data (Marwaha *et al.,* 2023). Economic barriers are significant, particularly for smallholder farmers. Even when the long-term benefits of phenotyping technologies are evident, the initial investment costs can be prohibitive. Financial subsidies, microfinancing, and government incentives may be necessary to make these technologies accessible at the farm level (Rose *et al.*, 2021). Large-scale farms and research institutions have had more success in implementing these technologies, but smallholder farmers need financial support to bridge the gap (Lipper *et al.*, 2017). Despite the challenges, successful case studies exist in regions such as India and parts of Africa, partnerships between agricultural research institutions and local farmers have enabled the adoption of drone-based phenotyping to monitor crop health. This has improved water use efficiency and increased yields (Chawade *et al.*, 2019). Research collaborations have shown how tailored solutions, paired with strong farmer education programs, can overcome many of the integration barriers (Balota and Oakes, 2017).

Data Management and Interpretation: Phenotyping systems generate massive amounts of data that require efficient management, interpretation and storage solutions. With the advent of high-throughput phenotyping (HTP) platforms, the volume and complexity of the datasets have increased exponentially. Managing these large datasets presents challenges in terms of storage capacity, computational power, and the ability to extract meaningful insights.

HTP platforms generate multispectral, hyperspectral and 3D imaging data as well as environmental and sensor data, which results in terabytes of information per growing season (Tong and Nikoloski, 2021). The effective management of these datasets requires high-performance computing (HPC) and cloud-based solutions which can process large-scale data in real time (Fiorani and Schurr, 2019). Image processing algorithms are often used to analyze large sets of visual data from various sensors and cameras. However, the quality of the output relies on the precision and accuracy of these algorithms, as even minor discrepancies in sensor calibration or environmental factors can lead to errors in the final analysis (Singh *et al.*, 2021). Data collection is just the first step, proper curation and storage are essential for long-term use. Phenotypic data needs to be organized into databases that can be easily accessed and queried by researchers, breeders, and farmers (Dwivedi *et al.*, 2020). The sheer volume of data makes it difficult to maintain without specialized tools and infrastructure, leading to the development of centralized platforms such as the European Plant Phenotyping Network (EPPN) and the Integrated Breeding Platform (IBP), which provide shared resources for data management and dissemination (Daviet *et al.*, 2022).

Sharing large datasets across institutions and countries is critical for advancing crop research and breeding programs. Open access to phenotyping data facilitates collaboration and speeds up the development of new crop varieties. However, data sharing is hampered by several factors, including the lack of standardization in data formats, which makes it difficult for different systems to interpret and exchange information (Chenu *et al.*, 2018). Data collected by different HTP systems or field phenotyping platforms might be incompatible due to variations in measurement protocols or sensor technologies (Hu and Schmidhalter, 2023). To address these challenges, efforts have been made to develop standardized protocols and metadata structures for phenotypic data sharing. Initiatives such as MIAPPE (Minimum Information About a Plant Phenotyping Experiment) have been established to provide guidelines for data sharing, helping researchers and breeders to collaborate more effectively (Papoutsoglou *et al.*, 2020). Another challenge in data sharing is the proprietary nature of some phenotypic datasets, particularly in commercial agriculture. Companies may be reluctant to share data due to competitive concerns or intellectual property rights. To overcome this, public-private partnerships have been proposed to facilitate the sharing of non-sensitive data while protecting the commercial interests of the stakeholders (Pieruschka and Schurr, 2019).

In addition to the technical and logistical challenges of data sharing, there are privacy concerns related to the ownership and use of phenotypic data. Farmers and researchers may be wary of sharing data, especially when it contains information about crop yields, soil health, or farm management practices, which could be exploited by competitors or used for profit without their consent (Kotal *et al.,* 2023). The growing reliance on cloud-based systems for data storage also raises concerns about data security. Breaches in these systems could expose sensitive agricultural information, including proprietary breeding lines or field-level data on crop performance. Ensuring that phenotypic data is protected by robust security protocols, such as encryption and user authentication, is essential for maintaining trust among data providers (Kuriakose *et al.,* 2020). Legal frameworks surrounding data ownership and intellectual property rights are still evolving in the context of phenotyping. Clarifying who owns the data collected by phenotyping systems-whether it be the farmers, researchers or technology providers-remains a pressing issue that requires regulatory oversight (Lassoued *et al.,* 2021). Ensuring fair access to and control over data will be crucial for the continued growth of phenotyping as a tool for crop improvement and precision agriculture.

FUTURE PROSPECTUS, IMPLICATIONS AND DIRECTIONS

Advancements in Phenotyping Technologies: AI and machine learning are playing a transformative role in modern phenotyping. Enhanced predictive models and decision support systems are improving the efficiency of plant breeding programs by rapidly analyzing large datasets and predicting phenotypic traits based on environmental and genetic factors (Sahoo *et al.,* 2024). These advancements have enabled real-time monitoring of crops and early detection of stress responses (Centorame *et al.,* 2024). Deep learning models have been successful in identifying complex traits with high accuracy, reducing the time needed for manual phenotyping (Arya *et al.,* 2022). The integration of phenotyping with genomics holds significant promise for enhancing the precision of plant breeding (Shakshi *et al.,* 2024). By linking phenotypic data with genetic information, researchers can better understand gene-trait relationships, enabling the advancement of more resilient crop varieties (Mir *et al.,* 2019). This integration also facilitates genome-wide association studies, where phenotypic traits are mapped to specific genomic regions, helping to identify key genes responsible for desirable traits (Xiao *et al.,* 2022). To ensure the widespread adoption of advanced phenotyping technologies, it is crucial to develop low-cost systems, particularly for smallholder farmers in developing regions (Reynolds *et al.,* 2019). Recent innovations include handheld devices and smartphone-based applications that offer affordable alternatives to expensive imaging systems (Nguyen *et al.,* 2023). These systems democratize access to precision agriculture tools, empowering small-scale farmers to monitor crop health and make data-driven decisions without significant financial investment (Karunathilake *et al.,* 2023).

Role in Sustainable Agriculture: Advanced phenotyping technologies are playing a crucial role in promoting sustainable agriculture by refining resource use efficiency and reducing the environmental footprint of farming practices. These technologies empower farmers to better monitor crop health, optimize the use of water, nutrients, and other inputs, and reduce wastage, ultimately leading to more sustainable farming systems (Janni and Pieruschka, 2022).

One of the primary areas where phenotyping contributes to sustainability is in water management. By accurately measuring crop water use and stress responses, farmers can implement precision irrigation techniques, which minimize water use while maintaining crop yields (Thorp *et al.,* 2018). This is particularly important in regions fronting water shortage due to climate change and increasing agricultural demands. Similarly, nutrient management is another area where phenotyping can enhance sustainability. Real-time monitoring of plant nutrient status allows for the precise application of fertilizers, reducing the risk of over-application and nutrient runoff, which can lead to soil degradation and water pollution (Shi *et al.,* 2020). Integrating phenotyping with precision agriculture practices can help in reducing the environmental impact of excessive chemical use. Phenotyping also aids in the expansion of climate-resilient crops, which are critical for addressing the challenges posed by global climate change (Cvejić *et al.,* 2022). By identifying traits associated with resilience to extreme temperatures, drought, and pests, researchers can breed crops that require fewer inputs while maintaining high productivity, contributing to both ecological sustainability and food security (Bohra *et al.,* 2021).

Policy and Regulatory Considerations: As phenotyping technologies evolve, there are important policy and regulatory issues that need to be addressed, particularly around data ownership, standardization, and ethical concerns. With the growing use of phenotyping platforms, including drones and IoT devices, large amounts of data are being generated. The question of who owns this data is becoming increasingly significant. Farmers, researchers, and technology providers may have different stakes in the data, raising concerns about intellectual property rights and the commercialization of agricultural data (Lajoie-O'Malley *et al.,* 2020). Policies need to clearly define ownership rights, ensuring that farmers retain control over their data while allowing for the responsible sharing of information for research and development purposes. There is a lack of standardized protocols for data collection and analysis in phenotyping, which creates challenges in comparing results across different studies and technologies (Tomičić *et al.,* 2022). Regulatory frameworks should work towards developing industry-wide standards to ensure consistency and reliability in phenotyping data. Standardization will also facilitate the integration of phenotypic data with other datasets, such as genomic or environmental data, enabling more comprehensive analyses. The use of AI and automation in phenotyping raises ethical concerns, particularly around the potential displacement of human labor and the unequal access to technology. Smallholder farmers in developing regions may be left behind if policies do not promote equitable access to advanced phenotyping tools (Ryan, 2023). Additionally, the use of sensitive genetic data in phenotyping could lead to privacy breaches or misuse if not properly regulated (Stanghellini and Leoni, 2020). Ethical guidelines are needed to ensure that these technologies are used responsibly and do not exacerbate social or economic inequalities.

Future Research Directions: There are several promising areas for future research in phenotyping that can help address current gaps and explore new applications. Despite the significant advances in phenotyping, some gaps remain that need to be addressed there. Phenotyping for below-ground traits, such as root structure and function, lags above-ground phenotyping (Blanchy *et al.,* 2024). Research should focus on developing tools and methodologies for non-invasive root phenotyping, which is essential for understanding water and nutrient uptake and improving drought tolerance (Wasaya *et al.,* 2018). Additionally, current phenotyping systems are often expensive, limiting their accessibility to resource-constrained farmers. Developing low-cost, scalable systems should be a priority for future research (Thrash *et al.,* 2022). Future research should also explore new applications of phenotyping, such as its potential role in biodiversity conservation and ecosystem monitoring. By identifying and characterizing plant species based on their phenotypic traits, phenotyping could help monitor changes in biodiversity due to climate change or human activities (Karaca and Ince, 2019). Moreover, integrating phenotyping with precision agriculture tools such as drones and satellite imagery and decision making tools could enable large-scale environmental monitoring, offering insights into ecosystem health and sustainability (Sweet *et al.,* 2022). The possibility of low-cost AI driven phenotyping system can also be explored to benefit small and marginal farmers.

CONCLUSION

Phenotyping systems have evolved significantly over the past few decades, from traditional manual methods to high-throughput, AI-driven technologies. The integration of advanced imaging techniques, sensor technologies, and big data analytics has revolutionized how phenotypic traits are monitored and measured. Despite these advancements, several challenges remain, including technical issues such as data accuracy and consistency, economic considerations around the cost of implementation, and the need for better data management and interpretation. Moreover, compatibility with traditional farming practices and ethical concerns, such as data privacy and ownership, present additional hurdles. However, the future holds exciting prospects, with advancements in AI, machine learning and genomics integration promising to enhance the precision of plant breeding. The development of low-cost phenotyping systems also offers hope for smallholder farmers, allowing them to adopt precision agriculture without significant financial strain. The continued evolution of phenotyping technologies will play a necessary role in addressing most demanding challenges in global agriculture, particularly in ensuring food security amidst climate change and population growth. By enabling more efficient resource use, promoting sustainability, and accelerating the development of climate-resilient crops, phenotyping will be at the forefront of agricultural innovation. Moreover, as technology becomes more accessible, especially with the development of affordable systems for smallholder farmers, the gap between high-tech and traditional farming practices may narrow. This could lead to a more equitable agricultural system where all farmers, regardless of scale, can profit from scientific and technological advancements. In this way, phenotyping will continue to be a driving force in the future of global agriculture, contributing to a more sustainable, productive and resilient food system.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

The author declared that the following contributions is correct.
Rizwan Ul Zama BANDAY: Conceptualization, investigation, writing (original draft),
Mohammad MUZAMIL: Supervision, writing (review and editing),
Danish Gul: Methodology, validation,
Seemi LOHANI: Methodology, validation,
Sehreen RASOOL: Software, visualization,
Kezia RAJAN: Formal analysis, data curation,
Muzamil HAMID: Formal analysis, data curation.

ETHICS COMMITTEE DECISION

This article does not require any Ethical Committee Decision.

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