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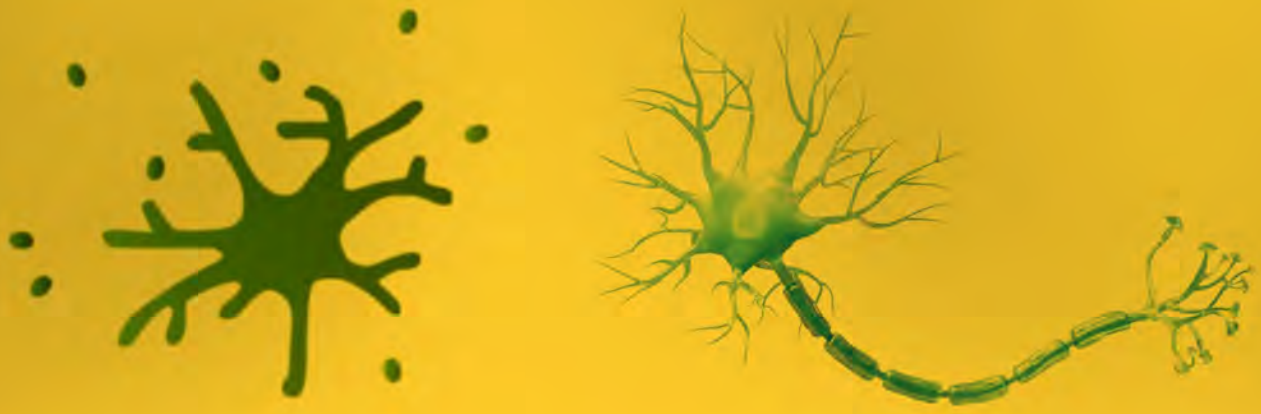

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RESEARCH ARTICLES

Bioscience Biotechnology Research Communications

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Have we tamed the coronavirus? May be yes,
as pandemics do not die, they can only be faded !

Science and technology has made it possible, in the shortest span of time, it has shown that with firm determination and international cooperation, we can win over the onslaughts of even the worst of the pandemics. COVID-19 is perhaps fading over now, due to our coordinated efforts worldwide. Though we have lost millions, in the two year period, partly due to the mishandling of the viral attacks and somewhat by our own follies and carelessness. Anyway lessons learnt from the past, always make us more stronger and determined. Let us now not relax and work on a better mode, as all is still not well yet. The almost taming of the virus and its cousins have indicated some of the concealed failures, on which we have to focus now. We have to be more vigilant, and even a bit of laxity can spoil the good work done. On societal and governmental parts, utmost care and caution is required on a long term basis.

On behalf of Bioscience Biotechnology Research Communications, we falter at words to express our deep sense of solitude and grief on the catastrophic events of the world wide pandemic, spanning over two years now. We pray for the strength to bear this universal calamity and come up with long lasting fortitude to eradicate it soon.

Biosc Biotech Res Comm is an open-access international platform for publication of original research articles, exciting meta-reviews, case histories, novel perspectives and opinions in applied areas of biomedical sciences. It aims to promote global scientific research and development, via interactive and productive communications in these areas, helping scholars to present their cherished fruits of research grown on toiled and tilled trees of hard work in life sciences. Being the publication of a non-profit academic Society for Science and Nature, Bhopal India, since 2008, *Biosc Biotech Res Comm* strongly believes in maintaining high standards of ethical and quality publication.

Quality publication is one of the ways to keep science alive, and good journals have a leading role to play in shaping science for humanity! As teachers, we have great responsibilities, we have to advocate our students to accomplish and show them the path to test their mettle in hard times to excel, especially in the post COVID 19 era. Science and its advocates will rise more to the occasion and will soon provide succor to the already grief stricken humanity.

Sharique A. Ali, PhD
Editor-in-Chief

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Socio-Demographic Influences on Perceived Stress and Mental Health During the COVID-19 Pandemic: A Multinational Study

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ABSTRACT

The present cross-sectional multinational study explores the effect of perceived stress during the COVID-19 pandemic on various socio-demographic factors, including gender, marital status, age groups, work abilities, social activities, and family relationships from Saudi Arabia, Canada, and Pakistan from April to July 2022. This cross-sectional study investigated perceived stress during the COVID-19 pandemic on various socio-demographic factors, work abilities, social activities, and family relationships. Online questionnaires were distributed to participants in Saudi Arabia, Canada, and Pakistan. Mental health indicators were assessed using the Work and Social Adjustment Scale. Spearman's correlation analysis revealed moderate impairment in working capacity and home management, with a significant effect ($r = .565$, $p < .001$). A significant association was observed between gender and engagement in leisure activities such as watching movies, indicating influence on mental well-being ($\chi 2(1, N = 295) = 6.83$, $p = .009$). Analysis of variance (ANOVA) showed significant differences in commitment to home management across four age groups (adolescent, young adult, adult, and mature adult) ($F (3, 294) = 3.887$, $p = .010$). Significant variations were also found in maintaining relationships with family members among different age groups ($F (3, 294) = 5.506$, $p = .001$). The findings underscore the association between anxiety and impairment in work and home management activities, and disruptions in leisure activities. These insights highlight the importance of targeted interventions to address mental health challenges during current and future healthcare crises. Further prospective studies are warranted to inform comprehensive intervention strategies and enhance resilience in future global challenges.

KEY WORDS: MENTAL HEALTH, STRESS, COVID-19, SOCIO-DEMOGRAPHIC FACTORS.

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INTRODUCTION

The advent of the COVID-19 pandemic has prompted unprecedented shifts in societal norms, influencing various facets of human behaviour and mental health worldwide. As the virus spread rapidly across the globe, governments implemented stringent measures such as lockdowns, social distancing, and quarantine protocols to curb transmission rates. These measures, albeit crucial for public health, have profoundly altered individuals' ways of life, posing significant challenges to their mental well-being and social interactions.

The interconnectedness between mental health and social behavior during the pandemic era has garnered increased attention from researchers and healthcare professionals. Numerous studies have elucidated the detrimental effects of prolonged isolation, economic strain, and uncertainty on individuals' psychological welfare (Torales et al., 2020). Furthermore, the disruption of daily routines, closure of communal spaces, and limited access to social support networks have exacerbated feelings of loneliness, anxiety, and depression among diverse demographic groups (Gagné et al., 2022).

Observations indicate that individuals aged 65 and above are more susceptible to infection due to compromised immunity and the presence of multiple co-morbidities (Monahan et al., 2020). Younger people are more likely to experience depression, stress, anxiety, and a negative outlook on life (Cohen et al., 2014). Moreover, it has been seen that the younger age group between 18-30 years has reported greater psychological distress during pandemics as compared to other age groups (McGinty et al., 2020). It has been seen that older age groups may also experience more social separation and solitude due to the COVID-19 Pandemic, which ultimately affects their mental health (WHO, 2020). Research indicates that the mental well-being of the global populace has been impacted during pandemics (Ahmad et al., 2020). There is another study reporting that females with poor self-related health status have shown medium to severe anxiety, depression, and stress ranks (Wang et al., 2020).

While the pandemic has underscored the importance of maintaining social connections, it has also catalysed the adoption of virtual platforms for communication and social interaction (Hards et al., 2022). Despite serving as a lifeline for many individuals during periods of physical distancing, the efficacy of digital mediums in mitigating social isolation and preserving mental health remains a subject of ongoing investigation (Abdulghani et al., 2022).Considering these developments, there is a compelling need for comprehensive, multicenter studies that examine the intricate interplay between mental health and social behavior amidst the pandemic. By elucidating the diverse manifestations of psychological distress, such research endeavors hold the potential to inform targeted interventions and policy initiatives aimed at promoting resilience and well-being on a global scale. Despite the growing body of literature examining the impact of the COVID-19 pandemic on mental health and social behavior, there remains a notable research gap in the understanding of these phenomena across diverse demographic groups and geographical regions.

Existing studies predominantly focus on specific populations or employ single-center methodologies, limiting the generalizability of findings and obscuring potential variations in the psychological responses to the pandemic. Therefore, through a collaborative approach, we aim to contribute valuable insights to the ever-expanding field of pandemic psychology and foster evidence-based practices for enhancing mental health outcomes in the post-pandemic landscape. Thus, we explored the effect of perceived stress during the pandemic on gender, marital status, different age groups, work abilities, social activities, and relationships with family members.

METHODOLOGY

This study employed a cross-sectional design to investigate the effect of perceived stress during the COVID-19 pandemic on various socio-demographic factors, work abilities, social activities, and relationships with family members. Participants were recruited from diverse demographic groups across North America, the Middle East, and Southeast Asia from April to July 2022. Inclusion Criteria: Individuals aged 18 years or older, fluent in the study language (English), and willing to provide informed consent. Exclusion Criteria: Individuals under the age of 18 or unable to comprehend English, as this could compromise the accuracy and reliability of their responses. Incomplete questionnaire submissions were also excluded from the final analysis. Additionally, individuals who did not provide informed consent or withdrew their consent at any point were not included.

Participants were recruited using an online questionnaire that was disseminated through social media platforms. The two primary platforms utilized in the study were Google Docs and WhatsApp. The Work and Social Adjustment Scale (WSAS) (Mundt et al., 2002), which is used to quantify functioning impairment, served as the foundation for mental health indicators in this study. Five items on this self-report form evaluated impairment in the five aspects of life: work, home management, social leisure activities, private leisure activities, and family and relationships. We examined the different influences of socio-demographic characteristics and impairment in functioning with WSAS as indicators of mental health among three different continents at one time.

The first part of the questionnaire includes questions on socio-demographic characteristics (age, gender, marital status, place of residence, religion, family size, occupation, level of education, average household income, family income minimum wage,

and living in an owned or rented house). The second part of the questionnaire was developed to understand the participants' experiences, such as the current and long-term effects of the current pandemic on mental health, pressures during the current situation, and activities that helped with mental well-being during the pandemic.

Descriptive statistics were computed to characterize the study sample in terms of demographic variables, perceived stress levels, work abilities, social activities, and relationships with family members. Inferential statistical analyses, such as chi-square tests and t-tests, were conducted to examine the associations between perceived stress and the previously mentioned variables while controlling for potential confounders. Subgroup analyses were performed to explore variations across gender, marital status, age groups, and work arrangements. Statistical significance was set at $p < 0.05$. This study received approval from the Institutional Review Board, College of Medicine, King Saud University, Riyadh, Saudi Arabia (Reference No. E-21-5897). All the participants provided informed consent before participation, and all data were anonymized to ensure confidentiality and privacy.

RESULTS AND DISCUSSION

Married participants (n=200) with postgraduate qualifications (n =114 (79.7%)) were the highest who participated in the study. The least number of participants based on marital status was divorced / widowed. However, they were highly qualified. Considering the educational level and marital status, a higher number of participants with high school certificates (n = 26 (55.3%)) were not married. [Table 1]

Table 1. Demographic characteristics

Characteristics Total No (295)		N (%)
Age	15-25	74 (25.1%)
	25-35	69 (23.4%)
	35-45	79 (26.8%)
	45+	73 (24.7%)
Gender	Males	101 (34.2%)
	Females	194 (65.8%)
Education level	High School	47 (15.9%)
	Undergraduate	105 (35.6%)
	Postgraduate	143 (48.5%)
Social Status	Single	79 (26.8%)
	Married	200 (67.8%)
	Divorced	9 (3.1%)
	Widowed	7 (2.4%)
Countries	Middle East	118 (40%)
	Southeast Asia	111 (37.6%)
	North America	66 (22.4%)
type of housing	Own house	153 (51.9%)
	Rented house	142 (48.1%)

The anxiety level of participants was observed across three continents. It was found that almost forty per cent of the people had uncertainty about their future and felt anxiety about their mental health. [Fig.1] When asked about feelings regarding the current COVID-19 situation, almost thirty per cent of participants expressed that whatever is happening is happening, and we must figure out how to proceed. [Fig.2]

Figure 1: Top concerns about the effects of the current pandemic on mental health.

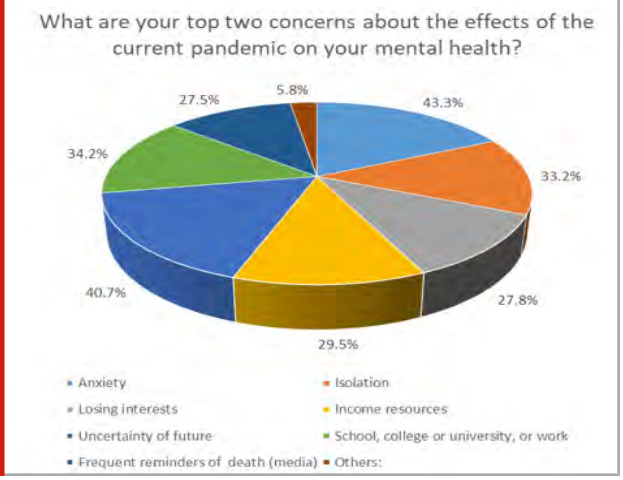
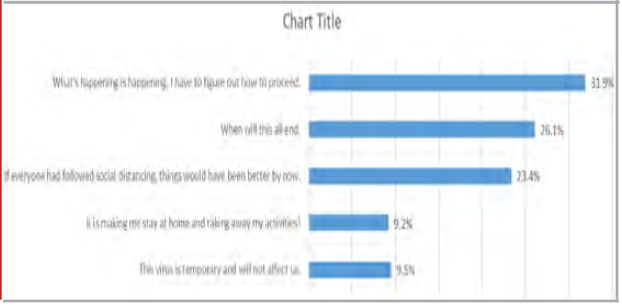


Figure 2: Feelings best described regarding the current COVID-19 situation.



A chi-square test of independence was executed to observe the association between gender and physical activity as a mental wellbeing cure during the COVID era. Men are more active in physical activity compared to women, and this gender difference was found to be statistically significant ($\chi^2 = 5.33$, $p = .021$). The participants also watched movies to get relief from pandemic depression. There was a significant association between watching movies and gender for mental wellbeing, ($\chi^2 = 6.83$, $p = .009$) However, listening to news about COVID from the media ($\chi^2 = 2.39$, $p = .122$) and adopting new hobbies ($\chi^2 = 2.05$, $p = .152$) had no significant association with restoring mental wellbeing (table 2). In our current study, we observed that during the COVID-19 pandemic, people primarily engaged in physical activities and consumed online content such as movies, compared to media exposure and adopting new habits for maintaining mental health.

This suggests that factors beyond media consumption or the adoption of new hobbies may not play a more significant role in restoring mental wellbeing during challenging times like the COVID-19 pandemic [Table 2].

Table 2: Maintaining the well-being of mental health through different activities.

Mental Wellbeing during COVID-19		Gender		X^2 (p)
		Male N (%)	Female N (%)	
Physical activity	No	63 (62.4%)	146 (75.3%)	5.33 (0.021)
	Yes	38 (37.6%)	48 (24.7%)	
Media Exposure	No	68 (67.3%)	147 (75.8%)	2.39 (0.122)
	Yes	33 (32.7%)	47 (24.2%)	
Movies / TV watching	No	80 (79.2%)	125 (64.4%)	6.83 (0.009)
	Yes	21 (20.8%)	69 (35.6%)	
New Hobbies	No	82 (81.2%)	143(73.7%)	2.05 (0.152)
	Yes	19 (18.8%)	51 (26.3%)	
Staying calm	No	57 (56.4%)	132 (68.0%)	3.88 (0.049)
	Yes	44 (43.6%)	62 (32.0%)	
Staying connected	No	51 (50.5%)	96 (49.5%)	0.027 (0.869)
	Yes	50 (49.5%)	98 (50.5%)	

Spearman's rho correlation between working capacity (more likely office working) and home management (shopping, washing, cooking, etc.) was moderately impaired, and the effect was significant (r =.565, p <.001). This showed that COVID has badly affected the working capacity of participants and their home management activities. Other variables, such as social activities and leisure activities, were also weakly impaired, and the disability was significant (r = .267, p < .001). There was a positive moderate effect on keeping the family relationships and leisure activities (r = .514, p <.001). In summary, it can be said that COVID-19 had a thorough effect on all types of activities. [Table 3].

Table 3: Correlation of Impairment in working capacity, home management, social activities, leisure activities, and family members' relationships because of the Pandemic.

Correlation	Working capacity	Home management	Social activities	Leisure activities	Family relationships
Working capacity	1				
Home management	0.565**	1			
Social activities	0.234**	0.312**	1		
Leisure activities	0.335**	0.495**	0.267**	1	
Family relationships	0.387**	0.458**	0.405**	0.514**	1

**. Correlation is significant at the 0.01 level (2-tailed).

The hypothesis tests whether the different age participants experienced the same workability or not. The participants were allocated into four age groups (Group 1: (15-25 years), Group 2: (26-35 years), Group 3: (36-45 years), Group 4: (46+ years). A one-way between-subjects ANOVA was run with four groups of participants as independent variables and their impairment regarding home management, retaining relations with family members, ability to work, and leisure activities as the Dependent variable [Table 4].

Similarly, the mean score of adult groups (n = 69, M = 4.25, SD = 2.654) was significantly different from the mature adult group (46+ years) (n = 73, M = 3.04, SD = 2.276; p = .024). However, there was no significant difference (P = .319) in the mean score of impairment in home management between age group 2 (young) and age group 3 (adult young) (n = 79, M = 3.48, SD = 2.782). [Table 4].

Table 4: Analysis of Variance (Pandemic impaired working capacity, home management, social activities, leisure activities, family relations).

Characteristics	Age	N	Mean(SD)	95% CI of the difference		ANOVA (df) F and P
				Lower	Upper	
Work capacity	15-25	74	3.77(2.6)	3.15	4.39	1.40 (0.241)
	25-35	69	4.03(204)	3.43	4.63	
	35-45	79	3.23 (2.1)	2.74	3.71	
	45+	73	3.51 (2.6)	2.89	4.12	
Home management	15-25	74	2.96 (2.2)	2.43	3.49	3.88 (0.010)
	25-35	69	4.25 (2.6)	3.61	4.88	
	35-45	79	3.48 (2.7)	2.86	4.10	
	45+	73	3.04 (2.2)	2.51	3.57	
Social activities	15-25	74	5.66 (2.1)	5.18	6.15	1.14 (0.332)
	25-35	69	5.77 (2.3)	5.21	6.33	
	35-45	79	5.14 (2.5)	4.58	5.70	
	45+	73	5.59 (2.1)	5.11	6.07	
Leisure activities	15-25	74	2.45 (2.3)	1.89	3.00	1.92 (0.126)
	25-35	69	3.30 (2.7)	2.64	3.97	
	35-45	79	3.10 (2.5)	2.52	3.68	
	45+	73	3.33 (2.4)	2.76	3.90	
Relations with Family members	15-25	74	3.76 (2.5)	3.17	4.34	5.50 (0.001)
	25-35	69	4.65 (2.8)	3.98	5.33	
	35-45	79	2.96 (2.6)	2.38	3.55	
	45+	73	3.40 (2.4)	2.82	3.98	

This multicenter research investigated the effect of perceived stress during the pandemic lockdown among adults across three continents, focusing on associations with demographic variables like gender, marital status, age, and family/work dynamics. It was found that around 50% experienced mental health-related anxiety. Married individuals reported more financial worries and future uncertainty than singles or divorcees. Another study found a greater decline in the emotional well-being of married people as compared to singles during this pandemic (Arafat et al., 2020; Sancassiani et al., 2024).

One of the studies reported that because of the pandemic, social life gets more restricted, and more confinement at home, which changed the whole family dynamics, so more arguments and less marital harmony (Abdulghani et al., 2023). Pandemic confinement affected family dynamics through more parental expectations from children, more expectations from either spouse, more domestic violence, or less mental harmony.

On the contrary, this study also showed that among married couples, the majority didn't perceive any long-term effect on their mental health, which seems to be a healthy outcome. Most married participants (n=200) held postgraduate degrees, which could be the reason that no long-term effects of this stress persist in their daily lives after the pandemic ended. Overall, male respondents (n =49; 48.5%) of different age groups considered no long-term effect of the pandemic on their mental health. This study also found that the pandemic had impaired home management among different age groups. Young adults (15–25) faced more isolation and distress. Similar findings were reported by studies elsewhere in which all these factors are associated with poor psychological well-being (Mushtaq et al., 2014; Alrashed et al., 2022; Mak et al., 2023).

Additionally, younger adults also experienced the impact of social isolation during the pandemic, even though middle-aged and older adults were already habitual living alone. Similar findings were reported by another study in which young adults reported poor mental health outcomes, probably due to high professional demand, more young parental duties, and a volatile economy (Arndt et al., 2006; Connor et al., 2022). It was also found that there was a significant difference in maintaining relationships with family members between the four age groups (P = .001). All age groups usually come across different types of social support. This mainly depends on the community and the social environment, and how they respond in times of need. There is a need to pay special attention and provide good and focused social support to

older age groups during the pandemic. Mental health resilience training helps to manage complex interactions and anticipate and manage stressors for vulnerable individuals, challenging any age boundaries. Fear during the pandemic significantly affected work, leisure, and home management (p=0.001). Changes in work hours, such as remote work during family time, disrupted both home and job life.

Another study has reported that fewer working hours due to pandemic restrictions (but more job obligations) cause a lot of disruption in routine work, with even fears of losing the job and ultimately more mental distress (Koh et al., 2020). This can be further explained in another report, in which 28% were affected by changes in their employment contract and 49% by changes in the quantity of work (Ahrendt et al., 2020). We also observed that in this study, social activities and leisure activities were significantly impaired, but the disability was significant ($r=267$, $p < .001$). This disability leads to a major impact on people's daily leisure routines, like exercising in gyms, playing video games, music, or using any other social media tools. Similarly, mixed patterns were seen in other studies found more engagement in leisure activities because of staying more time at home and having less travel time (Meyer et al., 2020).

Another study also showed significant variations in their exercise routine disruptions and highlighted a great deal of differences before and during the pandemic (Furman et al., 2023). Similarly, in a study in Qatar with 1114 people, it was reported that less physical activity led to a more sedentary lifestyle during the pandemic (Hidalgo-Andrade et al., 2021). So social isolation worsened anxiety, apathy, and detachment. This study reveals that passive social involvement with fewer leisure activities instigates a feeling of alienation; this ultimately affects health among all age groups during the pandemic. Longer-term studies are needed to assess the mental health burden and guide interventions for future crises.

LIMITATIONS

Limitations of this study include potential sampling bias inherent in online recruitment methods, reliance on self-report measures susceptible to social desirability bias, and the cross-sectional nature of the study design, precluding causal inferences. Additionally, the generalizability of findings may be limited by the study's reliance on convenience sampling and the exclusion of individuals with limited internet access or digital literacy.

CONCLUSION

The study found that the inability to do tasks related to working and managing the home was linked to anxiety symptoms. Moreover, certain activities were linked to degradation in social and private leisure activities during the pandemic. These findings highlight the broader impact of perceived stress on functional impairment across multiple domains. Longer-term prospective research is required to better understand the impact of psychopathology on various communities and social functioning, as well as to develop effective intervention techniques in response to the present and upcoming health problems.

Data Availability: All data will be available with the corresponding author on reasonable request

Conflict of Interest: None
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All authors agree to be accountable for all aspects of the work in ensuring that any questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval: This study received approval from the Institutional Review Board, College of Medicine, King Saud University, Riyadh, Saudi Arabia (Reference No. E-21-5897). All the participants provided informed consent before participation, and all data were anonymized to ensure confidentiality and privacy.

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Valorization of *Musa sp.* in the Production of an Alcoholic Beverage: The Banana Wine

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ABSTRACT

In the Republic of Congo, banana production is estimated at approximately 80,000 tons per year, mainly in regions far from the capital. Due to their climacteric nature, many bananas arrive overripe and soft after transport, becoming unattractive for consumption, leading to significant postharvest losses. Traditionally, *Musa* sp. is consumed fresh, leading to the rejection of a large quantity of overripe fruit. This study aimed to valorize *Musa* sp. by producing an alcoholic beverage, commonly known as "banana wine." The juice extracted from ripe bananas was stabilized, chaptalized, and fermented using *Saccharomyces cerevisiae*. Physicochemical parameters (temperature, °Brix, pH, titratable acidity, density, and alcohol content) were monitored throughout the fermentation. A sensory evaluation of the final product was also performed. Before fermentation, the banana must had a temperature of 29°C, 23°Brix, pH 3.84, density 1.086, and 0% alcohol. After fermentation, the wine had a temperature of 18°C, 7.5°Brix, pH 4.0, density 0.993, and 12.56% alcohol. These parameters comply with international quality standards (WHO). The results demonstrate that bananas can be effectively converted into a quality alcoholic beverage, thus reducing post-harvest losses while offering a potential new agro-industrial product for Congo.

KEY WORDS: MUSA SP., BANANA WINE, FERMENTATION, VALORIZATION, PHYSICOCHEMICAL PROPERTIES, POST-HARVEST LOSSES

INTRODUCTION

Banana (*Musa* spp.) is one of the most important fruits for food and economy in many tropical regions. Despite high global and local production, a significant portion of the crop is lost during post-harvest stages (harvesting, transport, storage, and marketing), affecting food security and producers' incomes. In sub-Saharan Africa, post-harvest losses for fruits and vegetables can exceed 40% of production, requiring adapted value-added strategies (Kikulwe et al., 2021, Affognon et al., 2023). Recycling bananas and their by-products (flesh, peels, etc.) into higher-value products is a promising way to transform these losses into economic and nutritional opportunities. Several recent reviews have highlighted the nutritional and functional potential of banana by-products, as well as processing methods for obtaining juices and musts suitable for fermentation (Hossain et al., 2022; Memon et al., 2023).

Among fermented products, "banana wine" has been studied in several contexts. The process generally involves juice

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extraction, stabilization, chaptalization, and fermentation by *Saccharomyces cerevisiae*, resulting in an alcoholic beverage with acceptable physicochemical and sensory characteristics (Bukar et al., 2020; Ssemugabo et al., 2021). Recent work has also validated standardized protocols to evaluate the fermentation performance of S. cerevisiae strains and control the quality of fruit wines (Padilla et al., 2022).

However, the literature shows significant variability in alcohol yields and sensory profiles depending on the *Musa* species/variety, maturity stage, must treatment, and yeast strain used (Hossain et al., 2022; Ssemugabo et al., 2021). In the Congolese context, where bananas are produced mainly in rural areas far from the capital and where significant post-harvest losses have been reported, banana wine production represents an attractive way to reduce losses, create local added value, and diversify producers' outlets (Kikulwe et al., 2021; Affognon et al., 2023). The objective of this study is therefore to assess the technological feasibility and the physicochemical and organoleptic quality of a banana wine produced from *Musa* sp. cultivated in Congo, following a protocol for preparing the must (extraction, stabilization, chaptalization) and fermentation by S. cerevisiae.

MATERIAL AND METHODS

Plant Material: The plant material used in this study consisted of dessert bananas (*Musa* sp.) purchased at the main train station in Brazzaville, Republic of Congo (Figure 1). The fruits were selected based on their commercial maturity, with no visible signs of deterioration or fungal contamination.

Figure 1: Fruits of Musa sp

Extraction of the Must: The banana juice (initial must) was extracted according to the following steps:

- Fruit Cleaning: Washing with potable water to remove surface impurities.
- First Weighing: Determination of the total mass of the raw fruit.
- Peeling: Manual removal of the peel.
- Second Weighing: Net mass of the pulp obtained.
- Dividing: Slicing into small pieces to facilitate extraction.
- Filtration: Extraction of the juice by mechanical pressing followed by filtration through sterile cheesecloth.

The resulting must was immediately stabilized by the addition of potassium metabisulfite (50 mg/L) to limit enzymatic oxidation and microbial growth (Amerine & Ough, 1980).

Banana (*Musa* sp.) Wine Production Process: Wine production from banana (*Musa* sp.) follows a series of technological steps aimed at transforming the must into a stable, aromatic, and clear alcoholic beverage. Banana wine production followed the following steps:

1. **Stabilization:** To limit the growth of unwanted microorganisms while preserving yeast activity, the must is initially stabilized by adding metabisulfite. This treatment controls the microbial flora without inhibiting fermentation.

2. **Chaptalization:** Chaptalization involves adjusting the sugar content of the must by adding sucrose to achieve the desired alcohol content and improve the sweetness of the final product. The sugar level was measured using a refractometer, set at 23°Brix.

3. **Cooling:** The must, obtained after thermal extraction, is cooled to approximately 25°C. This temperature is optimal for initiating alcoholic fermentation, generally effective between 25 and 30°C.

4. **Pitching:** The must is inoculated with a previously rehydrated dry strain of Saccharomyces cerevisiae. A preliminary fermentation called "pied de cuve" is carried out to prepare the starter: 5g of yeast is incorporated into a portion of must at 25°C. The appearance of foam after 15 minutes confirms fermentation activity.

5. **Main fermentation:** The starter is then introduced into the main must, triggering alcoholic fermentation. This is carried out in 10L carboys equipped with aseptic bungs to allow the CO_2 to escape. The fermentable sugars are then converted into ethanol, with the release of carbon dioxide.

6. **First siphoning:** Once fermentation is complete, the wine is decanted to remove the lees deposited at the bottom of the carboy. This operation is carried out using a siphon.

7. **Secondary fermentation:** Malolactic fermentation (MLF) converts malic acid to lactic acid, thus reducing the wine's acidity. It takes place at a temperature of 20 to 23°C. This phase is less active due to the reduction in fermentable sugars and the inhibition of yeast growth by the already present alcohol.

8. **Second siphoning and first fining:** A second decanting is performed to remove new lees. The wine is then clarified by adding bentonite (0.7g per 3L), a fining agent that flocculates suspended particles.

9. **Third siphoning and second fining:** A second fining is performed to improve the wine's clarity. Two fining agents were tested:

 Sample 1: bentonite for the first fining, egg albumin for the second (4 to 8 eggs per 225L).
Sample 2: Bentonite used for both finings.
Albumin promotes the flocculation of fine particles through protein precipitation. The two samples were compared based on organoleptic criteria: color, odor, taste, and aromatic persistence.

10. **Fourth siphoning:** This step separates the supernatant from the final sediment, resulting in a clear wine. It marks the final stage of the product, where aromatic adjustments can be made to impart a sensory signature specific to the manufacturer.

11. **Flash pasteurization:** The wine undergoes rapid pasteurization at 72°C for 28 seconds, ensuring its microbiological stability without altering its organoleptic qualities.

12. **Packaging:** The wine is bottled in pre-sterilized glass containers, guaranteeing hygiene and product preservation.

13. **Storage and aging:** The bottles are stored in a dry environment, away from light, and at room temperature. Aging allows the wine to develop its sensory characteristics and aromatic complexity.

Physicochemical Analyses: The physicochemical parameters of the must and wine were monitored at the various stages of fermentation:
Temperature: measured using a digital thermometer (AOAC, 2019).
Brix: determined by refractometry (AOAC, 2019).
pH: measured with a calibrated pH meter (AOAC, 2019).
Density: measured using a hydrometer.
Titratable acidity: determined by titration with 0.1 N NaOH and expressed as g/L of sulfuric acid equivalent (OIV, 2021).
Alcohol content: determined by distillation and densimetric measurement according to the official OIV method (2021).

Sensory Evaluation: A sensory analysis was conducted with a panel of 15 semi-trained judges. The characteristics evaluated included color, odor, taste, and aftertaste. A quantitative descriptive method was used to establish a sensory profile (Stone & Sidel, 2004). Samples were coded and randomly presented in ISO standard glasses.

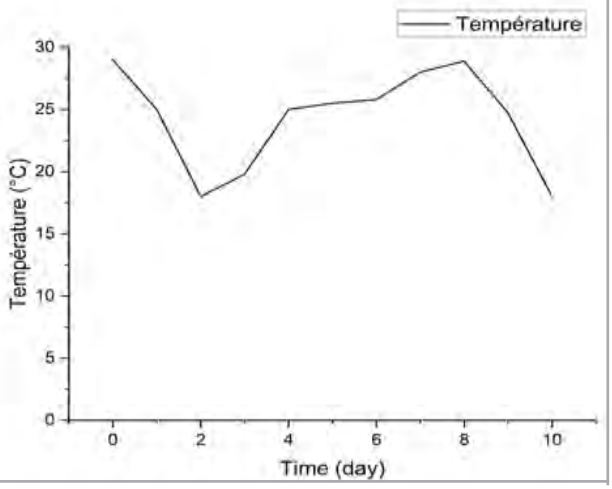
Statistical Analysis: All physicochemical measurements were performed in triplicate. Data were analyzed using OriginPro 8 software.

RESULTS AND DISCUSSION

Fermentation Process Monitoring Parameters: Temperature Changes During Fermentation

The temperature curve observed (Figure 2) over a 10-day period shows significant fluctuations, with a marked drop around day 3, followed by a gradual rise, and then a further drop around day 10. These variations reflect biochemical and microbiological phenomena typical of the fermentation process.

Figure 2: Temperature variation curve during alcoholic fermentation



The temperature evolution observed during the fermentation of banana must shows notable variations. On day 0 (29 °C), the relatively high initial temperature corresponds to the initial ambient conditions, before the influence of yeast metabolism. A sudden drop is observed on day 2 (18 °C), which can be explained by the loss of heat due to the thermal inertia of the medium and exchanges with the environment, before the fermentation becomes truly exothermic (Fleet, 2003).

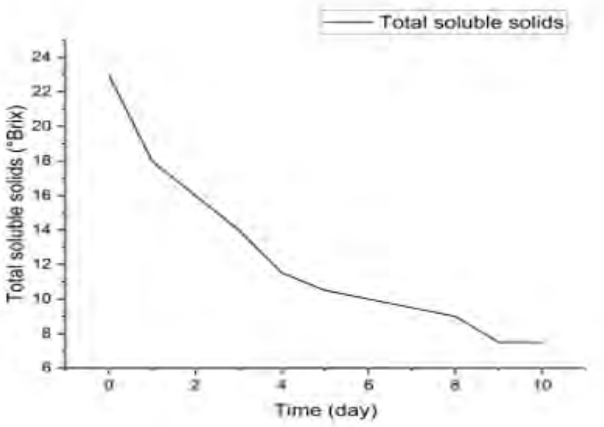
Between days 3 and 7, the temperature gradually increases, reaching 28.9°C on day 8. This increase reflects the intensity of the fermentative metabolism of Saccharomyces cerevisiae yeasts, which generate heat proportionally to the consumption of sugars (Bisson et al., 2017). This phase coincides with the peak activity of alcoholic fermentation, where fermentable sugars are converted into ethanol and CO_2 (Pretorius, 2000).

The decrease observed on days 9 and 10 (24.7°C then 18°C) corresponds to the end of primary fermentation. At this stage, substrate availability decreases, reducing the yeasts' metabolic activity and therefore heat production (Ribéreau-Gayon et al., 2006). Furthermore, the temperature drop can be accentuated by ambient environmental conditions and the gradual cessation of exothermic reactions (Walker & Stewart, 2016).

Overall, this thermal profile is consistent with the classic fermentation kinetics of tropical wines: initial thermal equilibration phase, exothermic phase linked to active fermentation, then decrease at the end of fermentation (Amerine & Ough, 1980; Fleet, 2003). Temperature monitoring is therefore a relevant indicator for controlling the speed and efficiency of fermentation (Jackson, 2014).

Evolution of total soluble solids (°Brix) during fermentation: The curve presented in Figure 3 shows a progressive decrease in the °Brix degree, going from approximately 23 °Brix on day 0 to almost 8 °Brix on day 10. This downward trend reflects the consumption of soluble sugars by the yeasts during alcoholic fermentation.

Figure 3: Variation of total soluble solids (°Brix) during fermentation



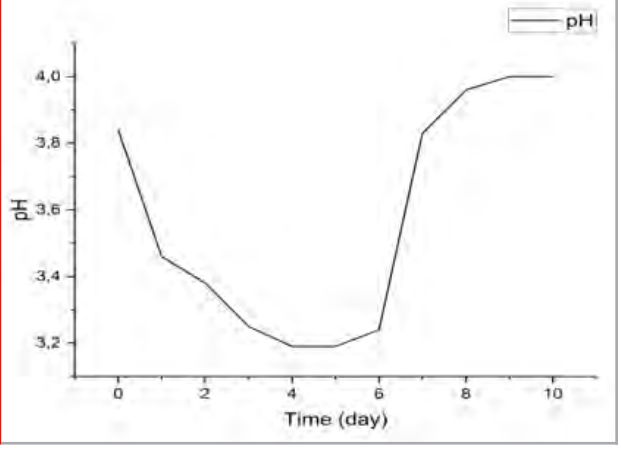
The results show a progressive decrease in total soluble solids (TSS) during fermentation, from 23°Brix on day 0 to 7.5°Brix on day 10. This decrease reflects the consumption of fermentable sugars (mainly glucose, fructose and sucrose) by yeasts, mainly Saccharomyces cerevisiae, for the production of ethanol and CO_2 (Fleet, 2003). The rapid drop in °Brix observed between day 0 (23°Brix) and day 4 (11.5°Brix) reflects an intense phase of alcoholic fermentation, corresponding to a high metabolic activity of the yeasts (Pretorius, 2000).

From day 5 onwards, the rate of decrease in °Brix becomes slower, reaching a near-stable value of 7.5 °Brix between days 9 and 10. This stabilization suggests the progressive depletion of available fermentable sugars and the end of the main fermentation, at which point yeast activity is reduced due to the reduction in substrates and the accumulation of ethanol (Bisson & Karpel, 2010). The reduction in total soluble solids is thus a key indicator for monitoring fermentation, confirming that the transformation of sugars into ethanol follows typical winemaking kinetics (Ribéreau-Gayon et al., 2006). The final value (7.5 °Brix) indicates the persistence of some residual sugars, which may contribute to the sensory profile of banana wine by influencing its sweetness and taste balance (Jackson, 2020).

pH Variation During Fermentation: Figure 4 illustrates the pH change in banana must (*Musa* sp.) over a ten-day fermentation period.

The results obtained show a progressive decrease in pH during the first days of fermentation, from 3.84 on day 0 to 3.19 on day 4, followed by a stabilization around 3.19–3.24 between days 5 and 7. An increase in pH is then observed from day 8, reaching 4 on day 10 (Figure 4). This trend is consistent with classical observations of alcoholic and lactic acid fermentation.

Figure 4: pH evolution



The initial decrease in pH is mainly attributed to the production of organic acids such as lactic acid and acetic acid by the activity of yeasts and lactic acid bacteria (Odăgeriu et al., 2005; Akin, 2008). This acidification promotes microbial growth while limiting the development of undesirable contaminants (Australian Wine Research Institute, 2023). The slight rise in pH observed at the end of fermentation could result from the partial consumption of organic acids or the formation of basic compounds (Atlas Scientific, 2023).These results highlight the importance of monitoring pH as a key indicator of fermentation progress and quality.

Variation of Titratable Acidity During Fermentation: Figure 5 illustrates the dynamics of total titratable acidity (expressed in g/L of H_2SO_4) over a ten-day process. A rapid increase in acidity is observed between day 0 and day 2, reaching a peak at approximately 1.2 g/L. This initial phase can be interpreted as a period of intense metabolic activity, often associated with microbial growth or primary fermentation, during which organic acids such as lactic or acetic acid are produced (Jay, Loessner, & Golden, 2005). Between day 2 and day 6, acidity remains relatively stable, suggesting a metabolic plateau phase.

This stability may indicate that the system reaches a balance between acid production and consumption, or that the dominant microorganisms have adapted their metabolism to the environment (Tamang et al., 2016). Finally, a slight decrease is observed after day 6, stabilizing around 1.1 g/L on day 10. This decrease may be attributed to the consumption of acids by secondary microorganisms or to chemical reactions such as precipitation or neutralization (Fleet, 2003).

The evolution of titratable acidity is a key indicator in fermentation, food preservation, or biodegradation processes. In fermentation systems, rapid acidification is often desired to inhibit undesirable microorganisms and

promote beneficial species (Caplice & Fitzgerald, 1999). The stability phase observed here is typical of controlled fermentations, where environmental conditions (pH, temperature, substrate) are optimized to maintain constant microbial activity. The slight decrease at the end of the process may also reflect a transition to a maturation or product stabilization phase, as observed in vegetable or dairy fermentations (Marco et al., 2017). This dynamic is consistent with observations made in similar systems, where acidity reaches a plateau before stabilizing or decreasing slightly.

Figure 5: evolution of titratable acidity

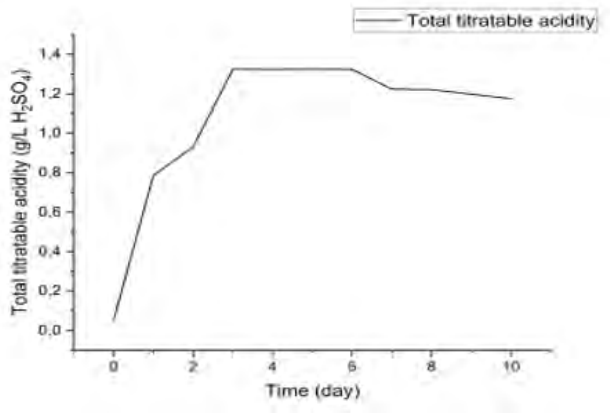
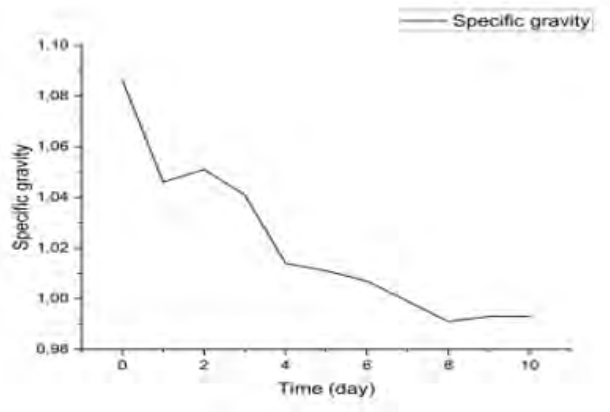


Figure 6: evolution of density



Variation in Wort Density During Fermentation: Figure 6 shows a gradual decrease in the specific gravity of a liquid, from approximately 1.08 on day 0 to 0.99 on day 10. This trend suggests a biochemical or physicochemical transformation of the medium, typical of fermentation or enzymatic degradation processes. Specific gravity is often used as an indirect indicator of the concentration of sugars or solutes dissolved in a liquid (Prajapati et al., 2014). A continuous decrease generally indicates the consumption of these substrates by microorganisms or enzymes, with the concomitant production of lighter metabolites such as ethanol, carbon dioxide, or organic acids (Steinkraus, 2004).

lactic acid fermentation, where sugars are transformed into lactic acid and other compounds, changing the composition of the medium (Tamang et al., 2016). Stabilization around 0.99 at Specific gravity kinetics is a valuable tool for monitoring the progress of a fermentation process without resorting to complex chemical analyses. It allows for estimating the rate of substrate consumption, the duration of the active fermentation phase, and the time of product stabilization (Caplice & Fitzgerald, 1999). In artisanal or industrial systems, this measurement is often used to determine the end of fermentation or to adjust production parameters.

In alcoholic fermentations, for example, specific gravity decreases as sugars are converted to ethanol, which is less dense than water (Fleet, 2003). This phenomenon is also observed in lactic acid fermentation, where sugars are transformed into lactic acid and other compounds, changing the composition of the medium (Tamang et al., 2016). Stabilization around 0.99 at Specific gravity kinetics is a valuable tool for monitoring the progress of a fermentation process without resorting to complex chemical analyses. It allows for estimating the rate of substrate consumption, the duration of the active fermentation phase, and the time of product stabilization (Caplice & Fitzgerald, 1999). In artisanal or industrial systems, this measurement is often used to determine the end of fermentation or to adjust production parameters.

The decline observed in this figure is consistent with typical controlled fermentation profiles, where the density drops rapidly in the first few days, corresponding to an exponential phase of microbial growth, followed by a slowdown as the substrates are depleted (Jay et al., 2005). This type of profile is also used in monitoring the fermentation of fruits, cereals, or dairy products, where specific gravity is correlated with the quality and stability of the final product (Marco et al., 2017).the end of the period may indicate that the majority of fermentable substrates have been consumed, and that the system reaches a state of metabolic equilibrium.

Specific gravity kinetics is a valuable tool for monitoring the progress of a fermentation process without resorting to complex chemical analyses. It allows for estimating the rate of substrate consumption, the duration of the active fermentation phase, and the time of product stabilization (Caplice & Fitzgerald, 1999). In artisanal or industrial systems, this measurement is often used to determine the end of fermentation or to adjust production parameters.

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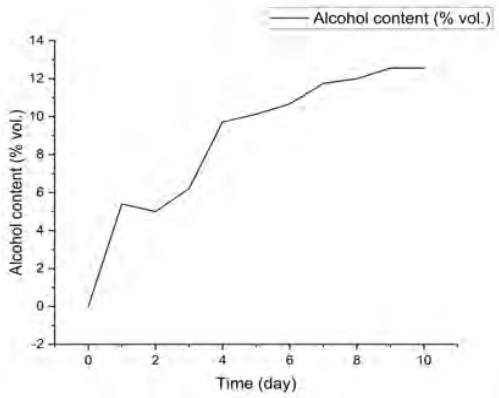
Changes in alcohol content during fermentation: Figure 7 shows a gradual increase in alcohol content (% vol.)

over a ten-day fermentation process. The alcohol content increases from 0% on day 0 to approximately 12% on day 10, with a marked upward slope, although punctuated by slight fluctuations. This trend is typical of alcoholic fermentation, where the sugars present in the substrate (often fruits or grains) are metabolized by yeasts, mainly *Saccharomyces cerevisiae*, to produce ethanol and carbon dioxide (Fleet, 2003).

Figure 7:



The initial phase (days 0 to 3) generally corresponds to the yeasts' adaptation to the environment, followed by an exponential phase of alcohol production (days 3 to 8), where the ethanol concentration increases rapidly. The stabilization observed around days 9 and 10 suggests that fermentable sugars have been largely consumed or that ethanol accumulation is beginning to inhibit microbial activity (Pretorius, 2000).

The evolution of alcohol content is a key indicator of fermentation performance and final product quality in the food industry, particularly in the production of wine, beer, and traditional fermented beverages. Efficient ethanol production depends on several factors: the yeast strain used, temperature, pH, initial sugar concentration, and the presence of nutrients (Bisson, 1999).

The observed curve is consistent with classical alcoholic fermentation kinetics, where ethanol production follows sigmoidal dynamics. Minor fluctuations may be due to variations in substrate availability or microbial interactions (Ciani & Comitini, 2015). A final concentration of approximately 12% vol. is typical of controlled fermentations, indicating good fermentation efficiency and near-complete sugar conversion (Zoecklein et al., 1995).

Table I: Analysis results compared to WHO standards

Settings	Banana wine	WHO standards	Interpretation
Temperature (°C)	18	20 - 28	Slightly below optimal range; may slow fermentation.
Sugar content (°B)	7,5	6 - 10	Compliant; adequate concentration for balanced alcoholic fermentation..
pH	4	3 - 4	At the upper limit; favorable to microbiological stability .
Titratable acidity (g/L of H2SO4)	1,76	/	High value, suggesting good protection against microbial spoilage.
Density	0,994	0,994 - 0,993	Conforms; indicates a well-completed fermentation
Alcohol content (°)	12	8 - 15	Conforms; concentration typical of a light to moderate wine.

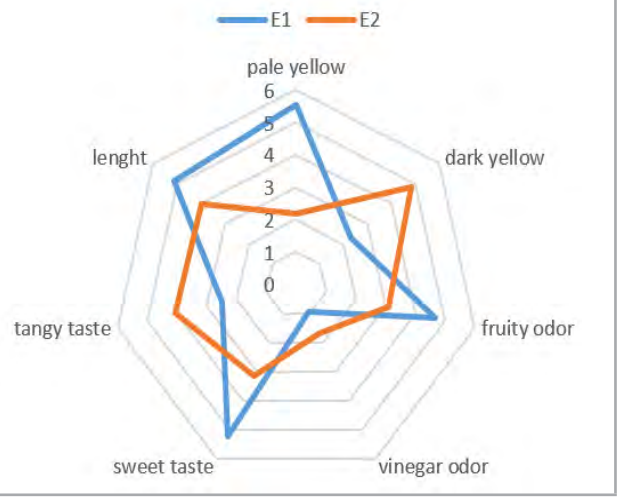
Comparative analysis of banana wine according to WHO standards: The fermentation temperature at 18°C, although slightly below the WHO recommendations (20–28°C), remains compatible with slow fermentation, often associated with better preservation of volatile aromas (Fleet, 2003). A lower temperature can, however, extend the fermentation time and influence microbial kinetics (Bisson, 1999).

The initial sugar content of 7.5°Brix is within the optimal range for efficient alcoholic fermentation, allowing ethanol production without excess sugar residue (Zoecklein et al., 1995). The pH of 4 is at the upper limit of standards, promoting product stability while limiting the growth of pathogenic microorganisms (Jay et al., 2005). The high titratable acidity (1.76 g/L H_2SO_4), although not specified in WHO standards, is generally desirable in fermented beverages to ensure good shelf life and balanced sensory perception (Caplice & Fitzgerald, 1999). The final specific gravity of 0.994 confirms that the majority of sugars have been converted to alcohol, which is consistent with an alcohol content of 12% vol., fully compliant with international standards for fruit wines (Steinkraus, 2004).

Comparative Sensory Analysis of Banana Wine: The figure presents a comparative sensory profile between two banana wine samples, E1 (blue line) and E2 (orange line), evaluated according to seven organoleptic descriptors: pale yellow, dark yellow, fruity odor, vinegar odor, sweet taste, tangy taste, and length in the mouth. Each attribute is rated on a scale of 0 to 6, reflecting the intensity perceived by a panel of tasters.

Color (Pale Yellow vs. Dark Yellow) E1 has a more pronounced intensity for pale yellow, while E2 is more associated with dark yellow. This difference may be related to fermentation duration or oxidation of phenolic compounds (Jackson, 2008). A darker hue is often correlated with advanced ripening or prolonged exposure to oxygen (Ribéreau-Gayon et al., 2006).

Figure 8: sensory profile



Fruity Odor E2 receives a significantly higher score, suggesting a better expression of volatile esters from fermentation (Swiegers et al., 2005). These compounds are essential for the sensory acceptability of fruit wines.

Vinegar odor: E1 exhibits a stronger intensity for this note, which may indicate slight acetic acid contamination or undesirable secondary fermentation (Drysdale & Fleet, 1988). A vinegar odor is generally perceived negatively and can compromise product quality.

Sweet and sour: E2 is distinguished by a higher perception of sweetness, while E1 is more sour. This contrast can be attributed to differences in residual sugar and organic acid content (Zoecklein et al., 1995). A good balance between sweetness and acidity is crucial for taste harmony.

Length: E2 surpasses E1 in terms of length, reflecting a longer aromatic persistence after tasting. This parameter is often associated with the complexity and perceived quality of the wine (Noble et al., 1987).

Length in the mouth: E2 outperforms E1 in terms of length, reflecting a longer aromatic persistence after tasting. This parameter is often associated with the complexity and perceived quality of the wine (Noble et al., 1987).

Overall, E2 presents a more favorable sensory profile, with higher scores for fruity odor, sweetness, and length, which are positive indicators of organoleptic quality. In contrast, the intensity of the vinegar odor in E1 could signal microbiological alteration or poor control of fermentation conditions (Fleet, 2003). These observations highlight the importance of controlling physicochemical parameters to ensure the sensory quality of banana wines.

CONCLUSION

This study demonstrates that the valorization of *Musa* sp. through alcoholic fermentation constitutes a viable technological approach for the production of quality banana wine. The physicochemical results obtained, including an alcohol content of 12%, a density of 0.994, and a pH of 4, comply with international standards (WHO), attesting to the control of the fermentation process. Furthermore, sensory analysis reveals a favorable organoleptic profile, particularly for sample E2, characterized by better aromatic expression and a pleasing finish.This valorization offers a concrete solution to reduce post-harvest banana losses in rural areas of Congo, while creating a promising agro-industrial outlet. It also paves the way for the diversification of banana products, with significant economic and nutritional potential. Additional studies on the product's stability, shelf life, and consumer acceptability would optimize its integration into local processing sectors.

Conflict of interest: The authors declare no conflict of interest

Data availability: All data are available with the corresponding author on a reasonable request

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Phytochemical Composition and Larvicidal Activity of *Sida acuta* Oil Extract Against *Aedes aegypti* Larvae

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ABSTRACT

Mosquito-borne diseases remain a major public health challenge globally, necessitating the search for eco-friendly alternatives to synthetic insecticides. Thus, this study investigated the phytochemical composition and larvicidal activity of *Sida acuta* leaf oil extract against *Aedes aegypti* larvae under varying treatments. Whole plant samples were collected in the morning (0600–0700 hours) and evening (1800–1900 hours), thereafter, identified, air-dried, then ground into fine powder, and extracted using n-hexane for a standing period of three days. Phytochemical constituents were determined using standard qualitative and quantitative methods. Eggs of *Ae. aegypti* mosquito were hatched in the laboratory during which the reared first-instar larvae of *Ae. aegypti* were exposed to varying concentrations of the oil extract at 12.5, 25, 37.5, 50, 62.5, and 125 ppb, respectively. Larvae knocked-down within the first hour exposure period were recorded immediately, and thereafter mortality was monitored at 24, 48, and 72 hours, respectively, post-exposure. Our qualitative analysis indicated high levels of alkaloids, steroids, and phenols, while flavonoids, tannins, and glycosides were present in low concentrations. Terpenoids were detected at low level in morning collection extract but were absent in evening extract. Anthraquinones and saponins were not detected in both time of day extracts. Quantitative screening confirmed the presence of flavonoids, phenols, tannins, alkaloids, saponins, terpenoids, cyanides, oxalates, and phytates. Larvicidal testing revealed lowest mortality rate of 1% for the morning extract in 50 ppb treatment and highest mortality rate of 3% for the evening extract in 62.5 ppb treatments after 72 hours exposure period. No significant difference ($P > 0.05$) was observed in the mortality rate in relation to treatments as well as between times of day. These findings demonstrate that *Sida acuta* leaf oil extract contains various bioactive phytochemicals. The larvicidal efficacy of *Sida acuta* against *Ae. aegypti* under the tested conditions was quite low which implies resistance but a more efficacious larvicidal activity maybe observed in the near future with increase in concentrations. In conclusion, *Sida acuta* leaf oil extract is a potential plant-based biopesticide for use in mosquito vector control.

KEYWORDS: *AEDES AEGYPTI, SIDA ACUTA*, PHYTOCHEMICALS, PLANT OIL EXTRACT, LARVICIDE, BIOSOLUTION, SUSCEPTIBILITY PROFILE.

INTRODUCTION

Plant-based pesticides are one of the most widely used biological mosquitoes and other vectors control methods because of their low cost, ease of availability, and eco-friendliness. They can also cause changes in the morphology, physiology, biochemical processes, and behaviour of different mosquitoes and other vectors life stages, indicating their significance in insect population control (Three thousand years ago, extracts from aromatic plants were employed as ectoparasite and anthelmintic repellents as well as to preserve harvested goods from pests, beginning a long history of the use of plant extracts for insect control. Based on their antibacterial properties and application in pharmaceutical goods, plant essential oils, particularly leaf

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oil extracts, have been employed, extensively (Scalvenzi *et al*., 2019, Selvakumaran *et al*., 2024).

Phytochemicals can be obtained from the entire plant or a specific part of it by extraction with various types of solvents such as methanol, ethanol, petroleum ether, water, and chloroform, depending on the polarity of the phyto-chemicals. They are also advantageous due to their ecosafety, target specificity, lack of record of insect resistance development, higher acceptability, and suitability for rural areas (Ngwamah & Naphtali, 2019). They are inexpensive, readily available, and environmentally beneficial, plant-based pesticides are a favourite among biological mosquito control techniques (Mwingira *et al*., 2020). Alkaloids, flavonoids, tannins, essential oils, and phenols are just a few of the Phytochemicals that have been reported to have insecticidal properties, whose effects vary depending on the plant species, mosquito species, geographical varieties and parts used, the extraction technique employed, and the polarity of the solvents utilized during extraction (Rodrigues *et al*., 2020; Souheila *et al*., 2020).

According to Aminah *et al*. (2021), *Sida* species possess a number of secondary metabolites, including those for alkaloids, flavonoids, coumarins, ecdysteroids, triterpenes, and tocopherols. Glycosides, steroids, and saponins are present in the ethanolic leaf extract of *Sida acuta* and amino acids, glycosides, terpenoids, steroids, flavonoids, and saponins are present in the petroleum ether leaf extract of *Sida acuta* in the study of the phytochemical screening of ethanol and petroleum ether leaf extract of *Sida acuta*, Burm. F. carried out to identify the secondary metabolites; however, neither of the Sida acuta leaf extracts contained either carbohydrates or tannins (Amitha & Joseph, 2019). Sida acuta is a powerful medication that has been used for centuries to treat a variety of conditions.

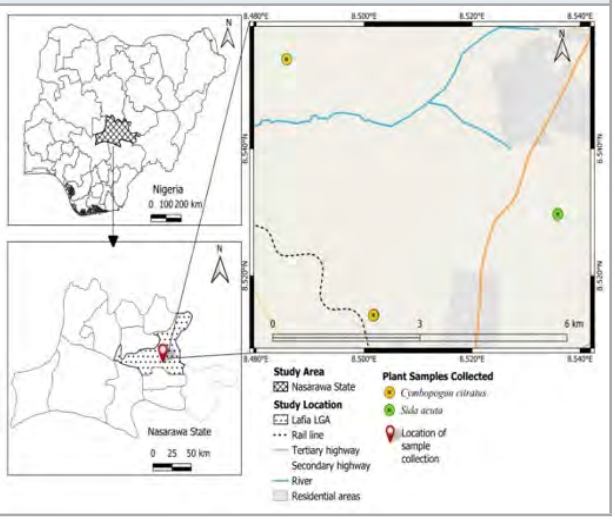
A review of the potential effects of the plant on the central nervous system and other diseases revealed that it contains phytochemicals with a variety of pharmacological actions, including terpenoids, β-phenethylamine, glutathione peroxidase, choline, vasicine, ephedrine, and cryptolepine (the main alkaloid of the plant), saponosides, coumarins, steroids (ecdysterone, β-sistosterol, stigmaterol, ampesterol), tannins, phenolic compounds (evofolin-A and B, scopoletin, loliolid, and 4-ketopinoresinol), polyphenol, sesquiterpene, and flavonoids (Tcheghebe *et al*., 2017). Consequently, based on the findings, it can be said that aqueous extract of *S. acuta* may have a wealth of medicinal qualities and may even be a unique medication (Abhishek *et al*., 2023).

Sida species, including *Sida acuta*, have been found to contain a variety of phytochemicals, including tannins, cardiac glycosides, and saponins, which may contribute to their potential biological activities. Research has shown that extracts from *Sida acuta* have larvicidal activity against Aedes mosquitoes, which are vectors of diseases like dengue and yellow fever. The plant's essential oil and hydrosol have also demonstrated strong larvicidal properties, and the plant's oil extract has been used to create silver nanoparticles, which showed promise as an environmentally friendly way to control vector mosquitoes (Krishnaveni et al., 2018; Ullah et al., 2018; Lwande *et al*., 2020; Shittu & Alagbe, 2020; Bassey *et al*., 2021; Usman & Abdulkarim, 2023). The phytochemical composition and mineral content of the plant suggest that it may have medicinal properties and potential uses. There is an urgent need to update the information status of the phytochemical screening of Sida acuta oil extracts and their larvicidal activity against *Aedes aegypti*, as there are not many studies online on that aspect. Therefore, this research evaluated the phytochemical composition and larvicidal activity of *Sida acuta* plant oil extract against *Aedes aegypti*.

MATERIAL AND METHODS

Study Area: This study was conducted in Lafia, Nasarawa State (Figure 1). Lafia is the State capital of Nasarawa, which also serves as one of the Local Government Districts in the State. Lafia lies between 8°29'N and 8°30'E latitude and longitude. The City has 330,712 residents overall, according to the 2006 population census (National Bureau of Statistics, 2006; Michael et al., 2016). The metro area population of Lafia in 2025 was 403,000, a 3.87% increase from 2024 (Abiodun, 2022).

Figure 1: Map of Lafia Metropolis in Nasarawa State showing the Collection Point (Created using QGIS Version 3.38.1)



The majority of the workforce of the state is employed in farming, and the products produced include cassava, yam, rice, corn, soybeans, asha, groundnuts, vegetables, sugar cane, and millet (Abiodun, 2022). The state is endowed with valuable mineral resources, including aquamarine, columbite and coal. Because most of the population works as farmers and cultivators of cash crops, they are more susceptible to diseases spread by mosquitoes as it makes room to plenty of mosquito breeding sites (Ombugadu *et al*., 2024).

Sample Collection: The leaves of *Sida acuta* (Wireweed) were sought for and collected from houses and schools around Lafia metropolis of Nasarawa state, Nigeria, in the morning (0600 - 0700 hours) and evening (1800-1900

hours) period of the day. The plant collected was identified and authenticated botanically in the Department of Plant Science and Biotechnology Laboratory, Federal University of Lafia and assigned the voucher number FUL/SC/PSB/H. LAB/0082. Firstly, the leaves were cleaned with running tap water to remove dust and debris from their surface. Thereafter, they were allowed to air dry at room temperature without exposure to sunlight using methods described by Nortjie et al., (2022) in the Department of Zoological Laboratory, Federal University of Lafia, Nasarawa State, Nigeria.

Figure 2: *Sida acutas* Plant (Field Photo)

Preparation of Plant Extracts: The leaves of *Sida acuta*, which were dried at room temperature, became brittle and easy to crush, then ground with a mortar and pestle to produce a fine powder (Osuagwu *et al*., 2021). Plant extracts were made in a 1:2w/v ratio, meaning that 91.1 g (*Sida acuta*) powder was prepared for every 125 ml of solvent. For the n-Hexane, 911.2 g of the powder from *Sida acuta* was weighed using a digital electronic laboratory scale (500G x 0.01G - SF-400C) and added to two different 2.5 L amber bottles that each held 1250 ml of n-Hexane respectively. The bottles were then left to stand for three days (seventy-two hours) with continuous stirring (Osuagwu *et al*., 2021).

The goal of the procedure was to release the soluble phytochemicals by breaking and softening the plant's cell wall (Nortjie *et al*., 2022). Using non-absorbent cotton wool and Whatman number (No.) 1 filter paper, the mixture solutions were filtered into conical flasks. The filtrates were concentrated by evaporating in a water bath at 60°C, weighed and recorded (Osuagwu et al., 2021). After being scraped off the conical flasks, the dried crude oil of the solvent was quantitatively transferred into sample containers and kept fresh in the refrigerator. Following extraction, determination of percentage yield and phytochemical analysis was performed on the crude oil leaf extracts to determine their active components. Determination of the Percentage Yield of Oil Extracts: The oil extract was weighed using a M-Metlar digital weighing balance (0.1 g – 3000 g) and the percentage yield of the oil extracts was calculated by the methods outlined by Mgbechidinma et al., (2023) as presented in equation 1.

$$\text{Percentage Yield} = \frac{\text{Weight of oil Extract (g)}}{\text{Weight of Crude Powder (g)}} \times \frac{100}{1} \ldots\ldots\ldots\ldots (1)$$

Percentage Yield of *Sida acuta* Oil Extracts

$$\% \text{ Yield for } \textit{Sida acuta} \text{ Oil Extract (Morning collection)} = \frac{6.9}{911.2} \times \frac{100}{1}$$

$$= 0.0076 \times 100$$

$$= 0.76\%$$

$$\% \text{ Yield for } \textit{Sida acuta} \text{ Oil Extract (Evening collection)} = \frac{7.4}{911.2} \times \frac{100}{1}$$

$$= 0.0081 \times 100$$

$$= 0.81\%$$

Qualitative Phytochemical Analysis of the Plant Oil Extract: The leaves of *Sida acuta* solvent oil extract were screened qualitatively for phytochemical constituents such as alkaloids, flavonoids, anthraquinones, steroids, saponins, phenols, terpenoids, tannins, and glycosides, using standard methods of analysis (Sofowora, 1993; Trease& Evans, 2002).

Test for Alkaloids: Few quantity of the each portion was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. Of the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second portion, Mayer's reagent was added and buff-coloured precipitate appearance was an indication for the presence of alkaloids (Sofowora, 1993).

Shinoda Test for Flavonoids: Few quantity of the each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease & Evans, 2002).

Borntrager's Test for Anthraquinones: About 0.2 g of each portion to be tested was shaken with 10 ml of benzene and then filtered. Five millilitres of the 10% ammonia solution was then added to the filtrate and thereafter shaken. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase was taken as the presence of free anthraquinones (Sofowora, 1993).

Test for Steroids: To 0.2 g of each portion, 2 ml of acetic acid was added, the solution was cooled well in ice followed by the addition of concentrated hydrogen tetraoxosulphate (VI) (H_2SO_4) carefully. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside (Sofowora, 1993).

Ferric Chloride Test for Phenols: About 0.5 of each portion was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of a phenolic hydroxyl group (Trease & Evans, 2002).

Knollar's Test for Terpenoids: A little of each portion was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of concentrated. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids (Sofowora, 1993).

Test for Tannins: The test extract was taken in water, warmed and filtered. 5 ml of filtrate was allowed to react with 1ml of 5% ferric chloride solution. If dark green or deep blue color is obtained, tannin is present (Dubale *et al.,* 2023).

Test for Saponins: One (1) ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Development of stable foam suggests the presence of saponins. Also, 1 ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins (Dubale *et al.,* 2023).

Test for Glycosides: Two (2) ml of extract, 3 ml of chloroform and 10% ammonia solution were added. Formation of pink color indicates presence of glycosides. Quantitative Phytochemical Analysis of the Plants Oil Extract: The quantitative phytochemical screening was conducted using the gravimetric analytical method of Khaled and Irani (2021) for the analysis of flavonoids, phenols, tannins, alkaloids, saponins, terpenoids, cyanide, oxalate and phytate. The methodological procedures employed for the gravimetric quantitative phytochemical analysis are shown below.

Determination of Flavonoids: One (1) g of the sample was weighed and repeatedly extracted with 100 cm_3 of 80% methanol at room temperature. The mixture was then filtered through filter paper into a 250 cm_3 beaker and the filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The % of flavonoid was calculated (Krishnaiah et al., 2009) as expressed in equation 2.

$$\% \text{ Flavonoid} = \frac{W2 - W1 \times 100}{W3} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: W_1 = Weight of beaker W_2 = Weight of beaker + filtrate W_3 = Weight of sample

Estimation of Total Phenol: The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five ml of the extract was pipetted out into a 50 ml flask, then 10 ml of distilled water was added. Two ml of NH_4OH solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 minutes for colour development. This was read at 505 nm. A standard was prepared from phenol stock standard (supplied by Sigma Chemical Company Limited England), this was slightly modified (Santhi & Sengottuvel, 2016).The concentration of the sample was determined by using phenol standard as shown in equation 3.

$$\frac{Cs}{As} = \frac{Cstd}{Astd} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where:- Cs = Concentration of sample, Cstd = Concentration of standard, As = Absorbance of sample, Astd = Absorbance of standard

Hence, concentration of sample (Cs) = $\frac{As \times Cstd}{Astd}$

Determination of Tannin: Tannin was determined using methods described by Horwitz and Albert (1996). This method was however slightly modified. About 2 g of sample was boiled with 300 ml of distilled water, diluted to 500 ml in standard volumetric flask and filtered through non-absorbent cotton wool. A volume of 25 ml of the infusion was measured into 250 ml conical flask and titrated with 0.1 N potassium permanganate (0.1 N) until pinkish colouration observed for at least 5 seconds. Potassium permanganate was standardized against sodium oxalate). The difference between the two titrates was multiplied by 3.0 to obtain the amount of tannin in the sample using equation 4.

0.1 ml potassium permanganate = 3.0 mg tannin ..... (4)

Determination of Alkaloids: We weighed 5.0 g of each sample and dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hours before filtering. The filtrate was evaporated to one-quarter (1/4) of its original volume. Some drops of concentrated ammonium hydroxide (NH_4OH) was added so as to precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper and washed with 1% NH_4OH solution. The precipitate was dried in filter paper in the oven at 60°C for 30 minutes and reweighed. From the weight difference obtained (i.e. weight of paper plus precipitate minus weight of empty filter paper),

$$\% \text{ Alkaloid} = \frac{W2 - W1 \times 100}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where: W = weight of sample W_1 = weight of empty filter paper W_2 = weight of paper plus precipitate

Test for Saponin: Saponin content: Total saponin content (% yield) was determined by gravimetric method as described by Kaur et al., (2015). The methanolic extracts from each plant (1 g in 10 ml) were macerated for 24 hours and then partitioned in water and n-butanol (1:1 ratio) solution. This solution was poured into the separator funnel and kept for 2 hours. The upper n-butanol layer was separated and the solvent was evaporated to obtain crude saponin extract as stated in equation 6.

$$\% \text{ Saponin} = \frac{W2 - W1}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Where:W = Weight of sample, W_1 = Weight of beaker, W_2 = Weight of beaker + saponin extract

Quantitative Determination of Terpenoid: Dried plant extract 100 mg (wi) was taken and soaked in 9 ml of ethanol for 24 hours (Malik, 2017). The extract after filtration was

extracted with 10 ml of petroleum ether using a separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoid content was measured by the formula in equation 7.

$$\% \text{ Yield of terpenoid} = \frac{wi - wf}{wi} x \frac{100}{1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

Where: wi = Weight of sample, wf = Weight of funnel + filtrate

Cyanogenetic Glycosides (Cyanide) Alkaline Titration: A portion of 10-20 g was placed in number (No.) 20 sieve in 80 ml of Kjedahl flask, add approximately 200 ml of water and let it stand for 2 to 4 hours (Autolysis should be conducted with apparatus completely connected for distillation). Steam distill, collect 150 to 160 ml distillate in sodium hydroxide (NaOH) Solution (0.5 g in 20 ml water), and dilute to definite volume (250 ml). To 100 ml distillate (it is preferable to dilute to 250 ml and titrate 100 ml aliquot) add 8 ml 6 m ammonium hydroxide (NH_4OH) and 2 ml 5% potassium iodide (KI) Solution and titrate with 0.02 m silver nitrate ($AgNO_3$), using microburrete. End point is faint but permanent turbidity was easily recognized, especially against black background (Horwitz & Albert, 1996) as shown in equation 8.

1 ml 0.02 m $AgNO_3$ = 1.08 g HCN (1 Silver equivalent to 2 Cyanide) ..... (8)

Determination of Oxalate Content by Titration: Oxalate ions are extracted from the plant parts by boiling them with dilute H_2SO_4 (0.5 N). Then oxalate concentration estimated volumetrically by titrating the extract with standard potassium per-manganate ($KMnO_4$) solution $MnO_4^- + 5C_2O_{42}^- + 8H_+ \rightarrow Mn_{2+} + 10CO_2 + 4H_2O$. This method was slightly modified.One gram (1 g) of plant material was weighed in electric weighing balance and transferred to 30 ml of 0.5 N sulfuric acid (H_2SO_4) and was boiled in a water bath for 15 minutes. Then the extract was filtered with Whatman's No. 1 filter paper. Equal volume of deionised water was added. Then 10 ml of filtered extract was taken and 40 ml 0.5 N H_2SO_4 was added. Final 50 ml of mixture was heated to 60° C and was titrated against 0.05 N $KMnO_4$. The end point was determined by permanent appearance of light pink colour (Mishra *et al*., 2017) as expressed in equation 9.

1 ml of $KMnO_4$ = 2.24% Oxalate ..... (9)

Test for Phytate: Phytic acid was determined using a method reported by Yahaya et al. (2013). Four grams (4 g) of ground sample was soaked in 100 ml of 2% hydrochloric acid (HCl) for 3 hours and then filtered through two layers of filter paper, 25 ml of the filtrate was placed in a 250 ml conical flask and 5 ml of 0.3% ammonium thiocyanate (NH4SCN) solution was added as an indicator, 53.5 ml of distilled water was then added to reach the proper acidity. This mixture was titrated against ferric chloride (FeCl3) solution, which contains about 0.00195 g of iron per ml of FeCl3 solution until a brownish yellow colour that persisted for 5 minutes was obtained. The result was multiplied by factor1.95 to obtain phytate P. Phytate P result was multiplied by factor 3.55 to convert to phytate.

Preparation of Stock and Working Solution of *Sida acuta* Oil Extract: The stock solution was prepared using methods as suggested by WHO (2018), where 10 g of each oil extract (n-hexane) of both plants was weighed using an ADAM PW 124 (Max 120 g d = 0.0001 g) Electric Weighing Balance. The weighed extracts were added to 20 ml beaker then distilled water was added and allowed to stand for 1 hour, with occasional agitation. The suspension was subsequently transferred quantitatively into a 50 ml volumetric flask by filtering using Whatman No. 1 filter paper and the volume was adjusted to 50 ml by adding distilled water to make the stock solution of 100 mg/ml. From this stock solution, working solutions were prepared to obtain concentrations in parts per billion of 12.5 ppb, 25 ppb, 37.5 ppb, 50 ppb, 62.5 ppb and 125 ppb, respectively, a modification used from WHO (2020). The working solutions (diagnostic doses) were prepared using equation 10 by WHO (2018): $C1V1 = C2V2$ (10)

Where: C_1 = Stock concentration (beginning concentration), V_1 = Volume of stock required to prepare new solution, C_2 = Concentration of new or working solution (desired concentration), V_2 = Volume of new solution desired

Culturing of Aedes Eggs for Larvae: Prior to the hatching of *Ae. aegypti* eggs gotten from National Arbovirus and Vectors Research Centre (NAVRC), Enugu State, Nigeria, nutrient broth was prepared by extracting nutrient broth from Agar powder, by adding, mixing and completely dissolving 13 g of nutrient agar powder (CM0001B) in 1 L of distilled water and allowing the agar residue to settle to get our pure nutrient broth which was then poured into a conical flask and sterilized by autoclaving at 121°C for 15 minutes. Thereafter, the egg stripes were soaked into nutrient broth and allowed to stand for 12-24 hours in order for the eggs to hatch to first instar larvae. The larvae hatched were later fed by putting a pinch of finely powdered biscuit and yeast unto the surface of the water.

Preparation of Bioassay: The susceptibility profile of *Ae. aegypti* first-instar mosquito larvae bioassayed to six concentrations (12.5 ppb, 25 ppb, 37.5 ppb, 50 ppb, 62.5 ppb and 125 ppb) of the n-hexane *Sida acuta* oil extract and was tested using the bioassay method in accordance with the recommended and advanced protocol of the World Health Organization [WHO] (2020) for testing of mosquito larvicides. For each concentration's assay, four replicates were maintained, along with a positive and negative controls each concentration's assay. In each replicate, twenty-five larvae were put into each of four disposable transparent rubber bowls (150 mL capacity) that held 100 mL of distilled water. Using a syringe, 1 milliliter of the working stock solution for each diagnostic dose was dispensed into each set of rubber bowls.

The positive control consisted of 1 ml of ethyl ether with 1 ml of n-hexane/100 ml of distilled water, while the negative control consisted of 100 ml of distilled water alone. The rate at which larvae were knocked down were noted at 10, 15, 20, 30, 40, 50, and 60 minutes, respectively, and larval mortality was measured after exposure period of 24, 48, and 72 hours, respectively. A tiny needle was lightly placed to the larvae's belly to verify their mortality (WHO, 2018).

A Taylor 1523 digital indoor/outdoor thermo-hygrometer was used to measure the temperature and relative humidity conditions in the lab.

Test Analysis: The interpretation of the mortality rate of mosquito larvae based on World Health Organization (2018) is as follows:

i. Mortality rate between 98 – 100 % within the diagnostic time connotes susceptible.
ii. Mortality rate between 80 – 97 % suggests possible resistance.
iii. Mortality rate < 80 % indicates resistance.

Determination of Percentage Mortality. Non-mobile and moribund larvae were recorded as dead as presented in equation 11.

$$\% \text{ Mortality} = \frac{\text{Number of Dead Larvae}}{\text{Number of Larvae introduced}} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (11)$$

Statistical Analysis: R Console software (Version 4.4.2) was used to analyze the observed data that was obtained. Pearson's Chi-square test was used to compare larval percentage mortality rate in relation to treatments, plants and time of day, respectively. Level of significance was set at $P < 0.05$.

RESULTS

Phytochemicals Qualitatively Detected in *Sida acuta* Oil Extract: From the qualitative analysis done for *Sida acuta* oil extract gotten from the leaves showed a very high concentration (+++) of alkaloids, steroids and phenol were detected in both morning and evening periods collections followed by flavonoids, tannin, and glycosides which were present in low concentration (+) at both time of day (Table 1). Terpenoids was only detected in the morning period at a low concentration (+) whereas anthraquinones and saponin were not detected (–) at all in *Sida acuta*.

Table 1: Qualitatively Detected Metabolites in *Sida acuta* Plants Oil Extracts in Relation to Time of Day

	Time of Day	
Metabolites	**Morning**	**Evening**
Alkaloid	+++	+++
Flavonoids	+	+
Anthraquinone	-	-
Steroids	+++	+++
Saponins	-	-
Phenol	+++	+++
Terpenoids	+	-
Tannin	+	+
Glycosides	+	+

Key: +++ = Present in high concentration, + = Present in low concentration= Not Detected

Quantitative Output of Phytochemicals in *Sida acuta* Oil Extracts: Table 2 shows the quantitative results using gravimetric method of analysis. With the exception of Tannin (120.28 mg/L), phenol (3.01%) and phytate (1.36%) that were higher in the evening hour, all other metabolites were more in the morning period. However, comparative analysis for each of the metabolite measured in relation to time of day showed no significant difference ($P > 0.05$).

Table 2: Quantitative Screening of *Sida acuta* Oil Extracts in Relation to Time of Day

	Time of Day				
Metabolite	**Morning**	**Evening**	**$\chi 2$**	**df**	**P–value**
Flavonoids (%)	6.73	1.65	3.0795	1	0.07928
Phenols (%)	1.86	3.01	0.2716	1	0.6023
Tannin (mg/L)	105.16	120.28	1.0141	1	0.3139
Alkaloid (%)	8.53	7.38	0.0831	1	0.7731
Saponins (%)	0.16	0.07	0.0352	1	0.8511
Terpenoids (%)	25.59	21.16	0.4198	1	0.517
Cyanide (mg/100g)	2.51	1.72	0.1475	1	0.7009
Oxalate (%)	1.60	0.55	0.5128	1	0.4739
Phytate (%)	1.07	1.36	0.0346	1	0.8524

Susceptibility Profile of *Aedes aegypti* Larvae in Relation to *Sida acuta* Oil Extract Treatments: No mortality was observed among *Ae. aegypti* exposed to the morning period treatments (Table 3). A 1% mortality rate was recorded in the evening period at 12.5 ppb at 24 hours exposure time and differences in mortality rate in relation to treatment and time of day, respectively, was not significant ($P > 0.05$). Also, at 48 hours exposures period, a mortality rate of 1% was obtained from the evening period treatments for 12.5 ppb, 62.5 ppb and 125 ppb, respectively. Although at 72 hours exposure period, 1% mortality rate was recorded in the morning period treatments at 50 ppb concentration. A high mortality rate of 3% was obtained in the evening at 62.5 ppb treatment followed by 2% mortality rate each at 12.5 ppb and 125 ppb. However, differences in mortality rate across all treatments as well as time of day showed no significant difference ($P > 0.05$).

DISCUSSION

This study in overall, based on qualitative processing, found the presence of eight metabolites in *Sida acuta* oil extract at varying concentrations, in which steroids, phenol alkaloid, glycosides, tannin, and flavonoids were recorded in both morning and evening period (Table 1). Studies by Musa *et al.* (2020) have shown that *S. acuta* is one of the plants with the largest group of phyto-chemicals, with amazing effects in humans, leading to the development of a powerful pain killer medication. This work agrees with previous study by Muneeswari et al., (2019) who reported that *Sida acuta* oil extract had the presence of alkaloids, steroids, tannins, glycosides, volatile oils, phenols and flavonoids as active phytochemical constituents. Our finding slightly agrees

with other studies that revealed that tannins, alkaloids, saponin, flavonoids, steroids, phenols, terpenoids, and hydrogen cyanide were present in *Sida acuta* crude extract (Senthilkumar et al., 2018; Usman & Abdulkarim, 2023; Edward *et al*., 2023). This study disagrees with that of Idowu (2023), which revealed a high presence of saponin content and also the moderate presence of anthraquinoun. These components could be responsible for the wide usage of the plant preparations for the treatment of various ailments, including asthma, fever, migraine, cough, cold, ulcer, snake bites, urinary diseases, and female disorders, and exploration as antidiuretic, antifertility and sedative agent (Akilandeswari *et al*., 2010).

Table 3:Mortality Rate of Aedes aegypti Larvae in Relation to Oil Extract Treatments

Exposure Period (Hours)	Time of Day	% Mortality in Relation to Treatment (ppb)							χ2	df	P-value
		0	12.5	25	37.5	50	62.5	125			
24	Morning	0	0	0	0	0	0	0			
	Evening	0	1	0	0	0	0	0	6.00	6	0.4232
	χ2		1								
	df		1								
	P-value		0.3173								
48	Morning	0	0	0	0	0	0	0			
	Evening	0	1	0	0	0	1	1	4.00	6	0.6767
	χ2		1				1	1			
	df		1				1	1			
	P-value		0.3173				0.3173	0.3173			
72	Morning	0	0	0	0	1	0	0	6.00	6	0.4232
	Evening	0	2	0	0	0	3	2	10.00	6	0.1247
	χ2		2			1	3	2			
	df		1			1	1	1			
	P-value		0.1573			0.3173	0.08326	0.1573			

In this research, terpenoid was absent in the evening collection of *Sida acuta* which reflects that the metabolite is only present in the early hours of the day which agrees with the findings of Hassan et al. (2022). The absence of anthraquinone and saponin in this study is in agreement with the findings of Hassan *et al.* (2022) who reported that anthraquinone and saponin were absent in *Sida acuta* collected from Kawo, Kaduna North Local Government area of Kaduna State.

The quantitative phytochemical screening of *Sida acuta* is presented in Table 2. The result revealed high amounts of tannins, alkaloids and terpenoids with moderate level of phenol, flavonoids, steroids, phytate, oxalate, cyanide and saponin metabolites which corroborate with the study by Nwankwo et al. (2023). The rich composition of the plant leaf fraction in phenolic compounds, steroids, terpenoids, alkaloids, and saponins suggests a potent pharmacological profile. Studies by Farooq et al. (2022) have proposed a correlation between the phytochemical composition of plant materials and their pharmacological activities. Steroids are well known to modulate inflammatory responses via their interactions with specific intracellular receptors which translates into either trans-repression (negative) or trans-activation (positive) of inflammation-related genes (Timmermans *et al*., 2019).

Flavonoids elicit anti-inflammatory and analgesic potentials by reducing intracellular Ca_{2+}, thereby repressing and preventing the activation of phospholipase A2, whose activity produces the precursor molecules for the synthesis of a variety of inflammatory mediators (Yang et al., 2020; Waghole *et al*., 2022; Yang et al., 2022). Tannins are potent cyclooxygenase (COX) inhibitors with a notable propensity to suppress edemogenesis induced by phlogistic agents (Attiqet *al*., 2018). The rich phenolic composition of the leaf fraction also implies excellentbioactivities, as phenolics are known to exhibit COX-1-sparing activities (Tungmunnithum et al., 2018).

According to this research, the oil extracts of *S. acuta* exhibited very low larvicidal activity against *Ae. aegypti* larvae, possibly due to very low start of dosage at part per billions (ppb). The low mortality rate of larvae in all exposure periods for *Sida acuta* treatment across concentrations for both morning and evening sessions is consistent with the findings of Ombugadu et al. (2020) and Pam et al. (2021), who reported a substantial variation in the mortality rate

of *Anopheles* species larvae across aqueous extract doses in Lafia Local Government Area, Nasarawa State, Nigeria. Various facets of insect physiology can be affected by essential oils: in addition to interfering with the respiratory enzymes of the mitochondrial membrane, the neurological system of the insect itself, it may have deleterious effects on growth, development, and reproduction (Feroz, 2020; Nwokocha, 2024).

Whereas, the low mortality rate of larvae in all exposure periods for *Sida acuta* treatment across concentrations for both morning and evening sessions disagrees with that of Saharayaj, (2022) who recorded a high mortality with start of low dosage of fern plant oil extracts, also ferns such as *Pteridium aquilinum, Polypodium vulgare, Schizaea dichotoma*, and others frequently produce ecdysteroids, which can have a deterring, antifeedant (repellent) effect, or cause toxicity, interference, or alteration of oviposition. It displayed miticidal (*Oligonychus coffeae)* and *insecticidal (Helicoverpa armigera, Spodopteralitura*, and *Helopeltistheivora*) properties. When ecdysteroids are consumed in greater quantities, monophagous insects that feed on them experience developmental abnormalities but would sooner starve to death than oligophagous insects (Saharayaj, 2022).

Sida acuta leaf hydrosol and volatile oils both exhibited variable degrees of larvicidal activity at various doses and exposure times according to reports from other authors who have shown that *S. acuta* contains phyto-chemicals that are effective in controlling insect pests, corroborating its insecticidal activity. Additionally, its ethanol extracts performed in a competitive manner against the target insect when used in conjunction with the synthetic insecticide, cypermethrin (Gadewad & Paedeshi, 2018; Babatunde *et al*., 2021). The various properties and uses of *Sida acuta* plant extract in traditional medicine can be attributed to its bioactive constituents, which include alkaloids like vasicine, ephedrine, and cryptolepine (the main alkaloid in the plant), saponosides, coumarins, steroids (ecdysterone, β-sistosterol, stigmaterol, ampesterol), tannins, phenolic compounds (evofolin-A and B, scopoletin, loliolid, and 4-ketopinoresinol), polyphenol, sesquiterpene, and flavonoid (Nalini *et al.*, 2021).

A variety of phytochemicals were found in the plant extracts utilized in this investigation and numerous substances that bear resemblance to these phytochemicals have been detected in different plant extracts and have demonstrated efficacy against a range of insects that cause disease (Torawane *et al*., 2021). Against *Anopheles stephensi*, the volatile oil and hydrosol oil of S. acuta shown significant in vitro cytotoxicity, with LC50 values of 106.40 mg/L - 80.00% killing and 101.22 mg/L - 70% killing at 500.00 mg/L, respectively. *Sida acuta* contains chemicals known as insect pheromones, namely tritetracontane and hemeicosane. The former is efficient against *Aedes aegypti*, the mosquito vector, and the latter is a major component of *Coleus aromaticus* essential oil, which shown considerable larvicidal activity against *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* (Amarasinghe *et a*l., 2020; Njoku *et al.*, 2021).

CONCLUSION

The phytochemicals present in the qualitative and quantitative measurements at caring concentrations were responsibile for the cause of *Ae. aegypti* death at the maximum rate of 3%.. This deaths could be explained by the ability of the n-hexane to solubilize a greater variety of bioactive compounds found in plant materials, as well as its relatively high polarity, this facilitates the dissolution of both polar and non-polar compounds, faster extraction rates, and greater stability of extracted compounds. Moreover, its lower boiling point makes evaporation easier and leads to concentrated extracts with higher potency, emphasising the importance of carefully selecting the right solvent for each extraction. Higher concentration of this plant should be explored.

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Homology Modelling and Evaluation of a Conserved Domain in *Oryza sativa* RING Finger Protein 45, a Putative E3 Ubiquitin Ligase

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ABSTRACT

The OsRFP45 identified in *Oryza sativa* has been identified as a negative salt-stress regulator. Its functions have been studied previously by overexpression and knock-out lines, but it has not been experimentally validated in structural form. This study provides a homology-based three-dimensional structural prediction of OsRFP45, an E3 ubiquitin ligase discovered in *Oryza sativa*, based on a matching template using SWISS-MODEL.Based on the existing literature, the locus Id of the OsRFP45 was identified. The locus Id was searched on NCBI to obtain the FASTA file of the identified protein. NCBI BLAST was conducted and it was analysed that the gene responsible for the production of this protein is highly conserved among various species. The homology modelling of the RING domain of OsRFP45 was performed using a template which had the highest GMQE (Global Model Quality Estimate). The accuracy of the predicted structural model was validated by running the bioinformatics tool - ProCheck and obtaining the Ramachandran Plot and related parameters. According to sequence alignment, OsRFP45's conserved RING domain is highly comparable to proteins from the RING domain found in other plant species.The OsRFP45 RING domain's conserved structural characteristics align with classical C3HC4-type E3 ubiquitin ligase. The three-dimensional structure shows a compact RING-domain stabilised by conserved cysteine and histidine residues, indicating a zinc-coordinated fold required for E3 ligase function. Zero percent of the disallowed region, 85.5% of the core and 14.5% of the allowed region are concluded by the Ramachandran plot. The predicted structure is hence, confirmed as correct.

KEY WORDS: BLAST, CONSERVED-RING OSRFP45, HOMOLOGY MODELLING, HYPOTHETICAL PROTEIN, MULTIPLE SEQUENCE ALIGNMENT.

INTRODUCTION

Over half of the world's population depends on rice (*Oryza sativa L.*), which is also the most significant crop in Asian nations. Africa has also seen a significant increase in rice consumption recently (Seck et al., 2012). The major abiotic stress which is responsible for poor yield and quality of grain is salinity. The accumulation of salt in soil causes stress conditions, due to this 10% of global cultivable land is not available for growth.(Saleem et al., 2025). The current estimate of saline land globally is 800 million hectares, with the amount increasing by 1% to 2% annually (Munns and Tester, 2008). Crop growth and development are greatly impacted by soil salinization, which also depletes soil nutrients and breaks down the structure of soil particles (Kordrostami, Rabiei and Kumleh, 2017). Rising sea levels brought on by global warming exacerbate salinity stress, which lowers soil fertility and hinders crop development in coastal areas (Shrivastava and Kumar, 2014). Rice grain yield encounters a sharp decline due to various forms of unavoidable biotic and abiotic stresses (Dixit et al., 2020 ; Khush, 2005). Previous studies have concluded that there is a nearly 29.29% decline in rice yield in soils with high salt accumulation in contrast to non-saline soils (Oelviani et al., 2024).

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Since it is a glycophyte by nature, too much salt adversely impacts its growth. Studying how rice reacts specifically to ion build-up above the threshold level can help identify the main causes of stunted growth and low rice yield, as well as the potential for future mitigation of the same (Ghosh and Ali, 2016). A RING-v-type E3 ubiquitin ligase has recently been identified, called OsRFP45 (*Oryza sativa* RING finger Protein 45). Present on chromosome 6, it has been concluded that it plays a major role in the negative regulation of salt tolerance in *Oryza sativa*. (Choi, Kim and Jang, 2025).

Plants have established post-translational mechanisms for regulation to combat the various stresses encountered, and the ubiquitin–proteasome system (UPS) is an essential component of these systems. Attaching ubiquitin molecules to target proteins and designating them for 26S proteasome destruction is known as ubiquitination. E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase) are the three enzymes that control this process. Rice's ability to withstand salt stress has been connected to a number of E3 ubiquitin ligases with conserved RING domains. Regulation of plant stress responses and determining specificity for the substrate depend on E3 ubiquitin ligases. Based on their structure, E3 ubiquitin ligases are further grouped into single and multi-subunit divisions. U-box, homology to the E6AP C terminus (HECT), and Really Interesting New Gene (RING) are the different groups of single-subunit E3 ubiquitin ligases (Deshaies and Joazeiro, 2009, Lee and Kim, 2011, Choi, Kim and Jang, 2025).

The plant stress hormone widely associated with response to environmental stimuli is Abscisic Acid (ABA) (Yamaguchi-Shinozaki and Shinozaki, 2006; Zhu, 2002). It has been observed that cellular ABA levels rapidly increase under adverse circumstances, which include a variety of outcomes, comprising - restriction of growth, closing of stomatal pores, sugar accumulation and generation of proline in significant amounts. Abscisic acid synthesis and the stress response it mediates are regulated by distinct E3 Ligases (Ko, Yang and Han, 2006). RING E3 Ubiquitin ligases are present abundantly in eukaryotic organisms. Rice and Arapidopsis consist of 378 and 499 genes, respectively in contrast to *Saccharomyces cerevisiae* which involves, 47 genes that encode for RING-type E3. (Kraft *et al*., 2005; Mazzucotelli *et al*., 2006). OsRFP45, a v-type RING E3 ligase, was identified as a prospective regulator of salt stress responses because of its transcriptional modification as an outcome of abiotic stresses such as drought and increased salt accumulation(Choi, Kim and Jang, 2025).

Previous studies examined into the way OsRFP45 controls ROS build-up, Na+ and K+ homeostasis, and its control of gene expression associated with stress under salt-induced stress (Choi, Kim and Jang, 2025). However, OsRFP45's structural characterisation is still limited because there isn't a 3D structure that has been experimentally determined yet. In order to shed light on the conserved C3HC4-type RING finger domain of OsRFP45 and its possible function in modulating protein–protein interactions and E3 ligase activity, the present study provides an in silico analysis of its structure. Homology modelling was performed to build a predictive 3D structure of OsRFP45's conserved RING finger domain, with the goal of acquiring a better understanding of its structural properties and functional properties.

MATERIAL AND METHODS

Numerous proteins' sequences can now be ascertained by efficiently recognising, isolating, and sequencing genes owing to the development of molecular biology tools. The protein structure is usually determined by X-ray crystallography, followed by Nuclear Magnetic Resonance. However, due to the increase in research and development of genomics there is an inevitable gap between the scientifically confirmed 3D model structures and the protein sequences found. In Silico techniques offer a solution to this gap by providing more rapid, cost-effective, and practical outcomes. Using previously determined structures of related proteins as templates, homology modelling is a computational technique that predicts the three-dimensional arrangement of proteins based on their amino acid sequences. It is regarded as the most accurate of computational structure prediction techniques (Bishop et al., 2008; Muhammed and Aki-Yalcin, 2018).

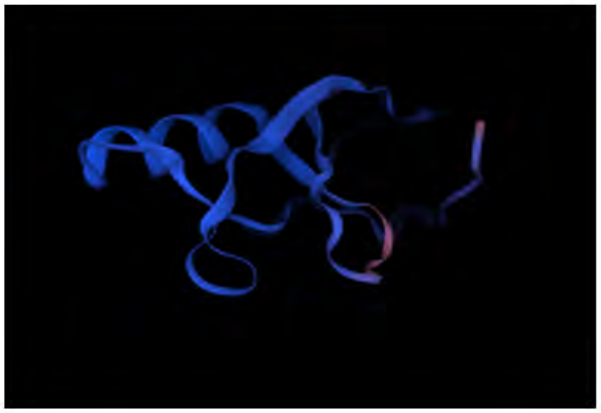
Sequence Retrieval: The OsRFP45 was identified as LOC_Os06g46366 (Choi, Kim and Jang, 2025). This was identified using the MSU Rice Genome Annotation Project, for cross-referencing the RAP-DB ID was obtained which was used to obtain the protein sequence from NCBI database. This retrieved the complete protein sequence, to get the exact RING sequence, InterProScan was used which annotated the exact sequence of the conserved RING region. The BLASTp alignment graphical summary confirmed that the RING finger domain, specifically the C4HC3-type protein was found out to be present at 241-289. This domain is involved in E3 ubiquitin ligase activity, typically mediating protein degradation. To accurately perform the homology modelling the range of (protein sequence) 231-300 amino acids was selected and saved as FASTA file.

The main phases involved in modelling a three-dimensional protein structure from its sequence: choosing a template to use, aligning the target and template, developing structural models, and assessing the models. SWISS-MODEL was chosen for homology modelling due to its reliability and continuous advancement throughout automated comparison modelling. The program is especially well-suited for producing precise 3D structures even in the absence of experimentally determined models since it makes use of large template libraries and advanced quality estimation metrics. In order to improve model accuracy and interpretability, its most current improvements include better protein assembly modelling capabilities and more biologically relevant template selection techniques (Waterhouse et al., 2018).

The FASTA file was first uploaded to SWISS-MODEL to find the matching template. The match having the highest GMQE (Global Model Quality Estimate) was AlphaFold DB model A0A0D2QDE7.1. A RING-CH-type domain-

containing protein which showed 0.89 GMQE. It resulted in 92.86% Seq. Identity. The Oligo-State of the template was monomer. This template was hence, chosen to accurately model the RING-domain of OsRFP45 protein.

Figure 1. Homology model of the RING finger domain of OsRFP45 (XP_015644308.1)



Model Assessment: The quality of the initial alignment and resulting sequence identity will have a significant impact on the model's final quality (Martí-Renom *et al.*, 2000). There are various methods for determining how accurate a model is, including geometrical inspections, empirical energy functions, and statistical grading. A protein structural model's utility is determined by its degree of precision. It should be attainable to produce a high-quality model structure, if a known protein matches more than 60% of its identity with the sequence being investigated (Chothia and Lesk, 1986). The protein template used (gene : A0A0D2QDE7_GOSRA, organism: *Gossypium raimnodii* (Peruvian cotton) (*Gossypium klotzschianum subspraimondii*) resulted in 92.86% identity, hence the homology model showed an accurate result.

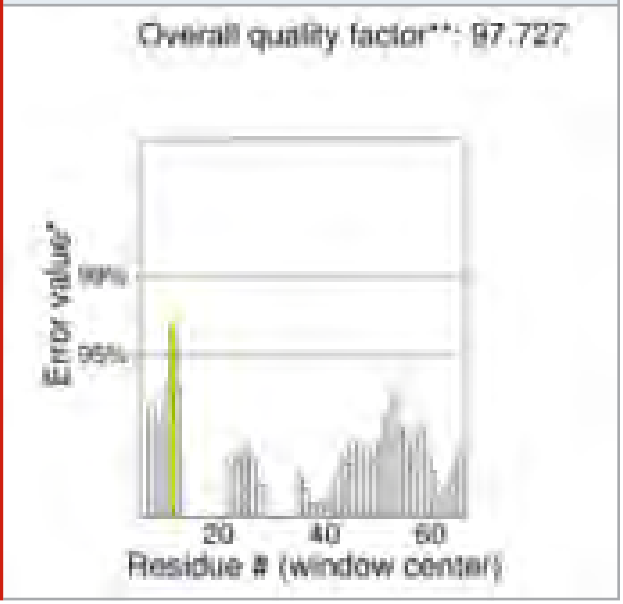
The modelled OsRFP45 domain was ran through several validation procedures through the SAVES server. ERRAT, which analyses non-bonded atom-to-atom interactions using a 9-residue sliding window, was used to evaluate the model's quality (Colovos and Yeates, 1993). It generates an overall quality factor based on comparisons to high-resolution structures; scores above 90% are suggestive of dependable models. It had an ERRAT quality score of 97.727%, which suggests that the predicted model had a high stereochemical quality. The ERRAT score confirms the accuracy of the model by indicating that there are no major areas of structural defects in the backbone and side chain environment. PROCHECK is a renowned bioinformatics tool for assessment of the reliability of stereochemical characteristics of three-dimensional structures of protein.

One of its primary outputs is the Ramachandran plot, which illustrates the spatial distribution of the backbone dihedral angles—phi (ϕ) and psi (ψ)—of all the amino acid residues in the protein structure. These angles determine the configuration of the polypeptide chain. The representation separates residues into most favoured, additionally allowed, generously allowed, and disallowed regions according to known steric constraints. A high proportion of residues in preferred locations indicates a reliable and accurately folded protein model.

The Ramachandran plot is a key validation step used to evaluate the modelled structure's overall reliability and stereochemical stability(Laskowski et al., 1993; Ramachandran, Ramakrishnan and Sasisekharan, 1963). The PSIPRED bioinformatics tool (Jones, 1999), which is well-known for its excellent accuracy in predicting α-helices, β-strands, and coil regions from the primary amino acid sequence, was used to further validate the secondary structure prediction of the modelled protein.

A further level of validation was provided by the PSIPRED output (Figure 3), which allowed a comparison between the secondary structural elements predicted and those seen in the homology model. The created three-dimensional model's dependability and appropriateness for additional investigations like docking or functional annotation are further enhanced by a high degree of agreement between the expected and modelled secondary structures.

Figure 2: ERRAT Overall Quality Factor – 97.727 %



RESULTS AND DISCUSSION

E3 ubiquitin ligases are characterized by a compact RING-domain in the expected three-dimensional structure, which is held in place by conserved cysteine and histidine residues that are responsible for coordination of zinc ions (Figure 1). The conserved RING domain's correct fold and functional conformation depend on this zinc-chelating motif. Using the PROCHECK tool, the model's stereochemical quality was evaluated. Zero percent of the disallowed region, 85.5% of the core and 14.5% of the allowed region are shown by the Ramachandran plot. The predicted structure is hence,

confirmed. According to this distribution, the model's stereochemical validity is supported and the anticipated structure complies well with established dihedral angle values (Figure 1).

Additionally, PSIPRED's projected secondary structure elements closely matched the modelled structure's observed elements, demonstrating a strong alignment of loops, β-strands, and α-helices. The structural accuracy of the homology model is further supported by this alignment. When combined, the PROCHECK and PSIPRED validation results verify that the modelled structure is accurate and suitable for additional functional or docking research.

Figure 3: PSIPRED Secondary structure

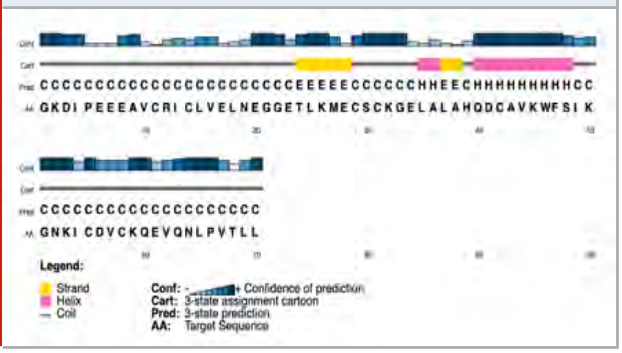
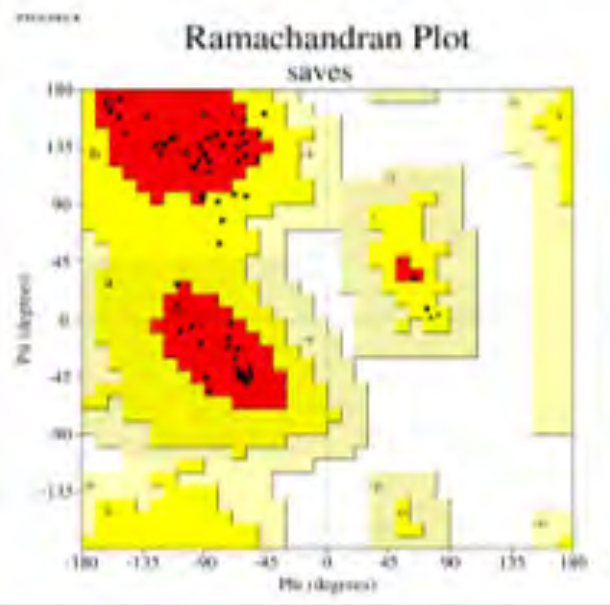


Figure 4: Ramachandran plot



CONCLUSION

In order to examine the spatial organization of conserved cysteine and histidine residues, the homology model of the putative RING finger protein OsRFP45 from *Oryza sativa* was built using SWISS-MODEL. The lack of experimentally determined structural data for OsRFP45 led to the pursuit of homology modelling. The study concentrated on the RING domain because the full-length protein's template alignment was not optimal. This region binds two zinc atoms necessary for structural integrity and ligase function. It is well-known for its chelating residues, which are mainly cysteine and histidine. Validation metrics showed a structurally sound model appropriate for additional docking and functional investigations, while the predicted 3D model showed a well-structured RING domain with conserved zinc-coordinating residues.

The conserved RING domain is represented by the 241–289 amino acid region, as validated by the BLASTp graphical summary. Furthermore, a secondary structure with short α-helices and β-strands scattered with coil regions was predicted by PSIPRED. These regions most likely represent flexible loops that support domain stability. When taken as a whole, OsRFP45's conserved structural characteristics provide compelling evidence for its designation as an E3 ubiquitin ligase. This model establishes the foundation for upcoming mutational and in silico docking investigations that may aid in elucidating OsRFP45's function in rice stress response networks.

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Conflict of Interest Statement: The authors declare that there is no conflict of interest.

Data Availability Statement: Data are available with the corresponding author on a reasonable request.

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Biosynthesis of Silver Nanoparticles from *Streptomyces rochei* and its Antibacterial Efficacy Against Bacterial Pathogens

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ABSTRACT

The unfolding of bacterial drug resistance poses a significant threat to public health globally, requiring novel and effective antimicrobial strategies. Knowing the necessity of this situation, the current study explores the biosynthesis of silver nanoparticles as potent antibacterial agents. The biosynthesis was explored by a green approach mediated by the endophytic actinomycete *Streptomyces rochei*, 16S rRNA sequencing helped identification of the actinomycete and assigned the GenBank accession number ON142045. The synthesized silver nanoparticles (AgNPs) were characterized for their formation by using various techniques, which included FE-SEM, EDS, FT-IR, UV-Vis spectroscopy, and DLS. UV-Vis analysis confirmed nanoparticle formation with an absorption peak at 400 nm. The FT-IR analysis shows existence of multiple functional groups such as O–H, C–N, and C=C, which explains biomolecules in nanoparticle stabilization. The biosynthesized AgNPs exhibited notable antimicrobial and bactericidal activity against both MTCC strains and clinically isolated Gram-negative bacterial pathogens. The above results show that the biosynthesized AgNPs possess significant potential as alternative antibacterial agents, offering a promising approach for combating multidrug-resistant bacterial infections. Utilizing endophytic actinomycetes for nanoparticle synthesis promotes environmentally sustainable methods while offering promising opportunities for biomedical use, particularly in antimicrobial treatments. This study highlights the effectiveness of biosynthesized silver nanoparticles in inhibiting bacterial growth, underscoring their potential role in addressing the ongoing challenge of antibiotic resistance through green nanotechnology-based solutions.

KEY WORDS: ANTIMICROBIAL ACTIVITY, BIOSYNTHESIS, BACTERIAL PATHOGEN, SILVER NANOPARTICLES, *STREPTOMYCES ROCHEI.*

INTRODUCTION

In the recent era of nanomaterials, metallic nanoparticles (NPs) are becoming increasingly significant due to their distinctive properties based on form, size, and dispersion (El-Baz et al., 2016; Mourdikoudis et al., 2018). Among these novel qualities are excellent physicochemical, electrical conductivity, catalytic, and mechanical stability (Srikar et al 2016, Crisan et al., 2021; Rania et al., 2021). Researchers have focused a lot of attention on different metal nanoparticles because of their effective physics, chemistry, and biological characteristics. Nanoparticles have proven useful in a variety of fields, including bioaugmentation of environmental contaminants, delivery of gene for the treatment and circumvention of genetic diseases, imaging, catalysis, and medical applications such as vaccine antigen administration, novel bioelectronics devices like nano-biosensors, metal optics, or enhanced drug delivery methods. Since multi-drug resistant bacteria are currently poorly managed, this is a serious worldwide problem. Therefore, the

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need for innovative antibacterial agents is essential. The lack of efficacious treatments for multi-drug resistance bacteria poses a serious worldwide threat. Finding new antimicrobial medications is therefore essential. In this regard, AgNPs have been suggested as a novel class of antibacterial agents.

Current treatments do not have the capacity to completely eradicate multi-drug resistant bacteria, posing a severe global threat (Wypij et al., 2018). Therefore, the need to develop innovative bactericides is very high. In many healthcare institutions, Gram-negative bacteria have been seen as the most frequent pathogens, there has been an upsurge in the incidence of nosocomial infection causing multidrug-resistance pathogens (Saeid et al 2011, Ahmed Elsadek Fakhr & Fayza Fathy, 2019; Rania et al., 2021).The antibiotic resistance among patients revealed the prevalence of methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta-lactamase bacteria, cephalosporin resistant *K. pneumoniae*, carbapenem resistant *K. pneumonia*, and multidrug resistant *P. aeruginosa, E. coli,* and *Acinetobacter baumannii spp*. (Ahmed Elsadek Fakhr & Fayza M. Fathy, 2019; Rania et al., 2021).

The antibacterial properties of AgNPs have led to their increasing popularity and even the suggestion that they represent a new class of antimicrobial drugs. As a result, numerous uses for Ag-NPs have been discovered, such as medical device coatings, household, wound dressings, and healthcare products, and cosmetics (Martínez et al., 2020). Majorly, two techniques for employed for synthesizing metal nanoparticles: "bottom-up" and "top-down." The methods involved are carried out by a number of chemical, biological, and physical processes. Many physical and chemical techniques are essential for the production of metal nanoparticles in view of their selectivity and capacity to produce monodisperse nanoparticles (Iravani & Zolfaghari, 2013).
The usage of a biological system is required for nanoparticles biological synthesis. Biosynthesis is a bottom-up strategy in which nanoparticles are formed as a consequence of metal reduction/oxidation and enzymes released by microbial systems and different metabolites from plants are responsible (Prabhu & Poulose, 2012). The bioproduction mechanism of AgNPs was reported according to which the enzyme of nitrogen cycle i.e., nitrate reductase is in charge of converting nitrate to nitrite. The nitrate reductase enzyme, is known to play a noteworthy role in the bioproduction of AgNPs. The silver ions are formed due to enzymatic metal reduction mechanism. Nitrate reductase reduces silver ions to metallic silver and therefore silver nanoparticles is formed (Karthik et al., 2014).

Many attempts have been made to synthesise metal nanoparticles utilising microorganisms and plants. In regards of biological synthesis, there is an entire list of bacteria and plants. Many kinds of bacteria, including *Bacillus sp.* and *Pseudomonas sp.*, are being used to reduce silver ions to silver nanoparticles. AgNPs have been manufactured using various actinomycetes which include *Streptomyces sp*. (Alani et al., 2012), *Streptomyces sp*. JAR1 (Chauhan, 2013), *Nocardiopsis sp*. MBRC-1 (Manivasagan et al., 2013), *Rhodococcus sp*. (Otari et al., 2012), *Streptomyces albidoflavus* CNP10 (Prakasham, 2012) *Streptomyces hygroscopicus* BDUS 49 (Sadhasivam et al., 2010), *Streptomyces sp*.VITPK1 (Sanjenbam et al., 2014), *Streptomyces rochei* (Kim et al., 1998), *Streptomyces sp*. BDUKAS10 (Sivalingam et al., 2012), *Streptomyces sp*. VITBT7 (Yingst et al., 1998), *Streptomyces sp*. I, *Streptomyces sp*. II, (Sukanya et al., 2013), *Streptomyces glaucus* 71MD (Tsibakhashvili et al., 2011), *Streptomyces aureofaciens* MTCC356 (Liossis et al., 1998) , *Streptomyces sp*. ERI-3 (Faghri Zonooz & Salouti, 2011), and *Streptomyces sp*. LK3 (Karthik et al., 2014).

Because of their promising bioactivities, such as antioxidant, antifungal, antibacterial, and anticancer effects, AgNPs have proved to be efficient metallic nanoparticles. The potential antimicrobial properties of these nanoparticles can be used in medical device industry to reduce microbial infections and prevent bacteria colonisation on medical equipment such as dental materials, catheters, vascular grafts, stainless steel materials, and human skin ("The Clinician and the Microbiology Laboratory," 2015). The present work involves the green biosynthesis of AgNPs by endophytic actinomycete *Streptomyces rochei* as an excellent alternative route, its characterization and evaluates the antimicrobial and anti-biofilm activities of the synthesized silver nanoparticles.

MATERIAL AND METHODS

Materials: Isolation of the actinomycete *Streptomyces rochei* was done from root tissue of *Bryophyllum pinnatum* (Lam.) Oken and the GenBank accession number ON142045 was assigned after 16S rRNA sequencing (Yadav et al., 2023). Himedia, Mumbai, India provided the chemicals used for preparing media (ISP-4 broth and NAM) and 2,3,5- Triphenyl tetrazolium chloride (TTC). Silver nitrate (AgNO3) was bought from Merck, Mumbai, India.

Silver nanoparticles biosynthesis: The endophytic actinomycete Streptomyces rochei was isolated (GenBank accession no. ON142045) (Yadav et al., 2023) and incubated in 250 ml of ISP-4 broth (pH 7.0) for 21 days at 29°C at 120 rpm. The obtained culture was filtered twice using a Whatman filter paper to remove any actinobacterial cell content, and the bio-extract was obtained. 200 mM AgNO3 was added to of above extract (1:1); to make a concentration of 100 mM of solution.

Centrifugation of the incubated solution was done at 10000 rpm at 4°C for 10 days, during which time it was surveilled for colour changes each day. After 10 days, pellet of the incubated solution was taken by centrifugation at 10000 rpm at 4°C. The pellet was then washed 3 times with ethanol and distilled water mixture and air dried. The obtained dried pellet was crushed in a mortar pestle to obtain fine powder and was further characterized. The detailed biosynthesis procedure of AgNO3 has shown in Scheme 1.

Characterization of biosynthesized silver nanoparticles: FESEM imaging: The biosynthesized AgNPs were ultra-sonicated in an ultrasonic cleaner (Aczet, Mumbai) with ethanol and drop casted on ITO glass plates to increase their

conductivity for visualizing using Field Emission Scanning Electron Microscopy (FESEM). At a magnification of 60.00 K X, FESEM images were recorded using FESEM (Carl Zeiss UHR, Gemini SEM 500 Kmat). Energy Dispersive X-Ray Spectroscopy (EDS) was also executed on the same sample to determine elemental composition.

UV-Vis spectroscopy: Using ethanol at a concentration of 1.0 mg/ml the biosynthesized AgNPs were ultra-sonicated in an ultrasonic cleaner (Aczet, Mumbai) and the genesis of AgNPs was analysed using UV-Vis Spectrophotometer (Thermo Scientific, Evolution 201) against ethanol as blank at a wavelength range of 300-700 nm.

Dynamic light scattering (DLS): The biosynthesized AgNPs were ultra-sonicated in an ultrasonic cleaner (Aczet, Mumbai) with double distilled water to for a clear aqueous solution and was analysed using the DLS method to estimate the hydrodynamic size and the zeta potential of AgNPs using zeta sizer pro (Malvern).

Fourier transform infrared spectroscopy: FT-IR spectra of the dried biosynthesized AgNPs sample was performed on an FT-IR spectrometer (Perkin-Elmer Spectrum IR Version 10.7.2), with KBr in the wave number region of 4000–400 cm−1. The identification of functional groups was done by collation of the spectral data recorded with the available reference database.

Antimicrobial activity screening: The antimicrobial activity screening of the biosynthesised AgNPs was conducted against MTCC and clinical cultures of four Gram-negative bacteria *(Escherichia coli, Proteus mirabilis, Salmonella typhimurium, and Pseudomonas aeruginosa)* . Every bacterial strain was cultivated on nutritional broth. In order to create fresh stock inoculum suspensions of the pathogenic strains, colonies from 24-hour cultures grown at 37°C were selected, and the colonies were then suspended in sterile saline solution (0.9% NaCl, w/v). By adjusting the optical density of pathogens, the turbidity was brought to the 0.5 McFarland standard or approximately 1×108 CFU/mL for bacteria. Bacterial cultures were inoculated on Nutrient Agar Media plates and wells were created with 0.1 mg/ml of the bacteria. Next, three copies of the silver nanoparticle were inoculated to all the wells and the incubation was done at 37 °C for 24 h and observed.

MIC, MBC method, and tolerance level: In ELISA plates, the lowest concentration of AgNPs that can stop bacterial growth known as minimum inhibitory concentration, or MIC was measured. A silver nanoparticle was used against the bacterial cultures of *Escherichia coli, Proteus mirabilis, Salmonella typhimurium*, and *Pseudomonas aeruginosa* in an ELISA plate and incubation was processed for 24 hours at 37 °C after adding a serial dilution by 2-fold ranging from 1 mg/ml to 0.0019 mg/ml to each well. To quantify the amount of inhibition, 2,3,5-Triphenyl tetrazolium chloride (TTC) was added after incubation. Both negative control and positive control with bacteria but no AgNPs were examined.

The least concentration at which bacteria are eliminated is the minimum bactericidal concentration (MBC) of AgNPs. The procedure involved inoculating a sample from every well in the previously conducted experiment onto a Nutrient Agar plate, followed by a 24-hour incubation period at 37 °C. The substance's bacteriostatic and bactericidal qualities are assessed in relation to the tolerance level. The material is bacteriostatic if the tolerance level is greater than 16, and bactericidal if it is ≤4. The tolerance threshold is calculated using the MBC/MIC method.

RESULTS AND DISCUSSION

In this work, the culture supernatant of fresh *Streptomyces rochei* was successfully used to manufacture AgNPs extracellularly, within 6 hours of incubation, the biosynthesis of AgNPs was mediated by the culture supernatant treated with 100 mM AgNO3. The reaction mixture's colour changed from yellow to a dark reddish-brown colour, signifying the creation of AgNPs. In contrast, neither the uninoculated media with AgNO3 as a control nor the culture supernatant without AgNO3 showed any colour change. After ten days, the colour remained constant, signifying that AgNO3 had entirely decreased and the reaction had come to a stop which is depicted in Figure 1.

Figure 1: Biosynthesized of nanoparticles: (a) control; (b) and (c) colour change observed after 10 days of incubation.

Scheme 1. Biosynthesis procedure of silver nanoparticles (AgNPs).

Because AgNPs are environmentally friendly, there has been a great deal of interest in their cost-effective biological production. Ag-NPs are surface-coated with various biomolecules from the *Streptomyces* supernatant, which increases their biocompatibility and safety. Consequently, intriguing uses of biological synthesis can be advantageous for the fields of biology and other related sciences.

At a magnification of 60.00 KX the FESEM images showed the nanoparticles formed were nanosized (1-100 nm) and the shape observed was spherical as depicted in Figure 2a. The EDS showed the elemental composition which confirms the existence of Silver shown in Figure 2b. At 400 nm, a sharp and wide UV-Vis band maxima peak was seen shown in Figure 3a, which is typical for AgNPs. The DLS represents the value of how the particle diffuses within water. Generally, the hydrodynamic size of a particle is larger than its original diameter. The DLS pattern showed the Z-average diameter of the Ag-NPs biosynthesized by *S. rochei* was 146.17 nm, and its Zeta potential was 0.4 displayed in Figure 3b&3c respectively. Multiple functional groups were seen on the surface of biosynthesized Ag-NPs was verified by the FT-IR analysis displayed in Figure 4. Results revealed distinct, strong absorption bands at 2921.27 cm-1 (O-H stretch), 1636 cm-1 (C=C alkenyl stretch), 1041 cm-1 (C-N stretch), and 594.10 cm-1 (wagging region), which indicated that phenols and alcohols were the capping agents. The increased ability of proteins to bond with metals and to create a surface layer on metallic AgNPs via their amide and aromatic group residues proves that they serve as a binder for metals.

Figure 2: (a) SEM Images of biosynthesized AgNPs; (b) Energy Dispersive X-Ray spectroscopy of biosynthesized AgNPs.

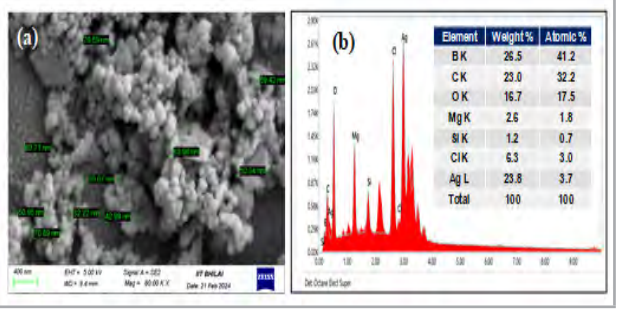


Figure 3. Characterization of biosynthesized silver nanoparticles: (a) UV-Vis spectra; (b) zeta size; (c) zeta potential.

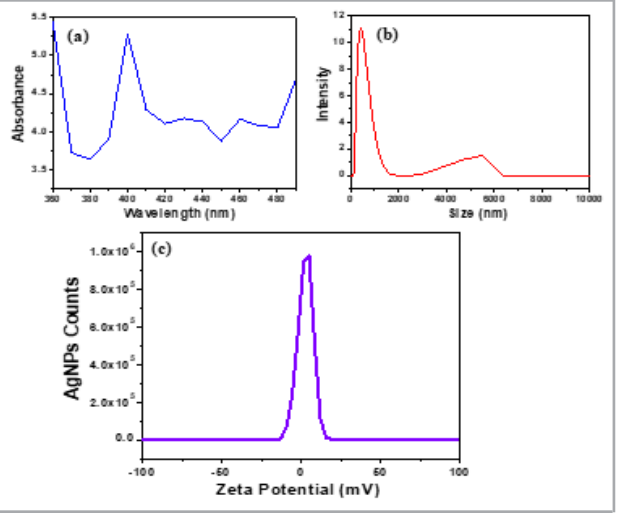
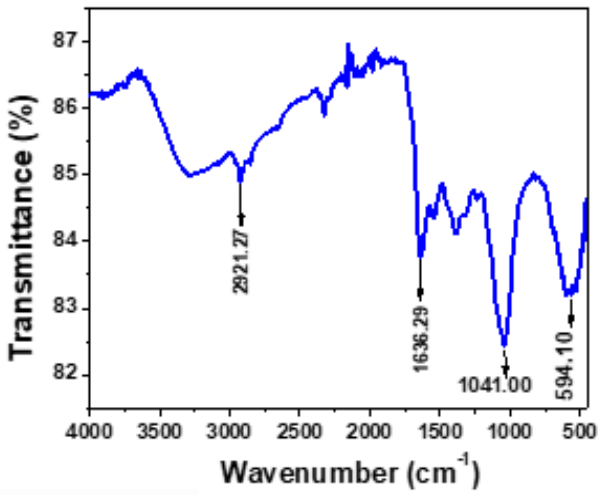


Figure 4: FT-IR Spectra of biosynthesized silver nanoparticles



Resistance mechanisms have been observed in bacteria for many generations; chemical antimicrobial treatments are limited in the current situation. When it comes to the problem of multidrug resistance, AgNPs are a great improvement over conventional chemical antimicrobial agents since metallic NPs are less likely than traditional antibiotics for bacterial resistance.

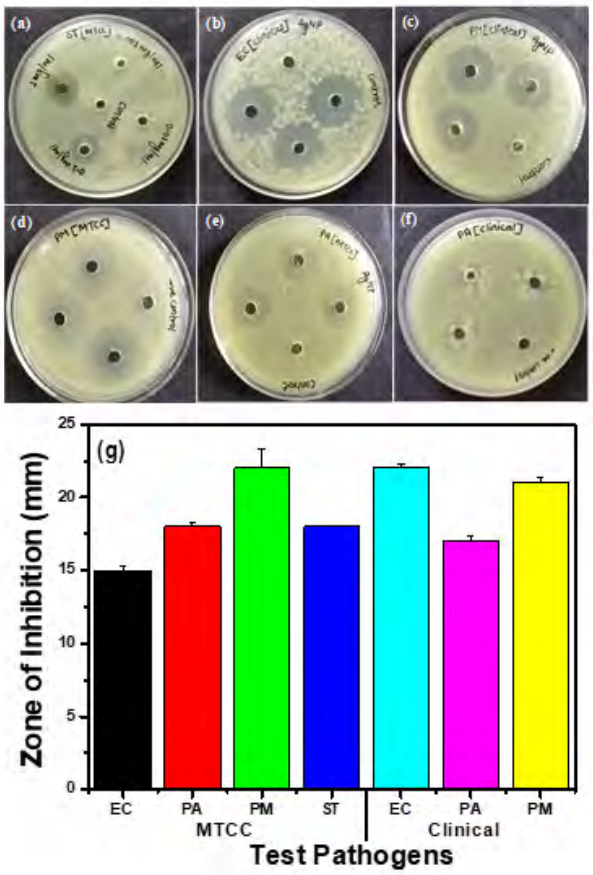
The biosynthesized AgNPs showed exceptional dose-dependent antibacterial effectiveness in MTCC and clinical cultures of numerous different Gram-negative bacteria, *Escherichia coli, Salmonella typhimurium, Proteus mirabilis, and Pseudomonas aeruginosa* shown in Figure 5(a-f). With an inhibitory zone diameter of 24 mm for *Escherichia coli* and 22 mm for *Proteus mirabilis* in the MTCC culture, antimicrobial activity was demonstrated by biosynthesized Ag-NPs against all pathogens shown in Figure 5 (g). MTCC cultures of *Escherichia coli* and *Salmonella typhimurium* exhibited the least activity, with inhibitory zone diameters of 14 and 18 mm, respectively. The biosynthesized Ag-NPs partially inhibited the advancement of the clinical strain of *Pseudomonas aeruginosa.*

To find out if the biosynthesized AgNPs have a bacteriostatic or bactericidal impact against tested pathogenic strains, the intensity of tolerance should be assessed. According to (Das et al., 2014), an antibacterial agent is classified as bacteriostatic when the ratio of MBC/MIC is ≥16 and bactericidal when the ratio is ≤ 4. According to National Clinical Committee for Laboratory Standards, if an agent reduces CFU/mL by 99.9% after a day of incubation in broth media it is deemed bactericidal. Also, if the microbe's MBC is 32 times or more greater than its MIC, it is deemed tolerant (Woods and Washington 1995).

Figure 6 (a-g) and Table 1 displays the Minimum Inhibitory Concentration (MIC) of silver nanoparticles against the MTCC and clinical cultures of *Salmonella typhimurium, Proteus mirabilis, Escherichia coli*, and *Pseudomonas aeruginosa*. 2,3,5-Triphenyl tetrazolium chloride (TTC)

for MIC measures the metabolic activity that bacteria are undergoing and reacts by turning red if metabolites are present. AgNPs' bactericidal action is indicated by absence of red colouring. The Minimum Bactericidal Concentration (MBC) of AgNPs against MTCC and clinical cultures of *Escherichia coli, Salmonella typhimurium, Proteus mirabilis, Pseudomonas aeruginosa,* and both positive and negative controls is displayed in Figure 7 (a-g) and Table 2.

Figure 5. Antibacterial activity of biosynthesized silver nanoparticles against (a) *Salmonella typhimurium* (MTCC-98), (b) *Escherichia coli* (clinical), (c) *Proteus mirabilis* (clinical), (d) *Proteus mirabilis* (MTCC- 425), (e) *Pseudomonas aeruginosa* (MTCC-424), (f) *Pseudomonas aeruginosa* (clinical) and (g) Graphical representation of antibacterial activity silver nanoparticles against Gram negative pathogenic bacteria.



While the negative control shows no bacterial growth, the positive control shows bacterial growth in the absence of AgNPs. *Salmonella typhimurium*, *Escherichia coli*, and *Proteus mirabilis* have tolerance levels between 1-4, indicating bactericidal activity, while *Pseudomonas aeruginosa* has a tolerance level of >16, indicating bacteriostatic activity.

Figure 6. Minimum inhibitory concentration of biosynthesized silver nanoparticles against (a) *Escherichia coli* (MTCC- 40), (b) *Escherichia coli* (clinical), (c) *Proteus mirabilis* (MTCC- 425), (d) *Proteus mirabilis* (clinical), (e) *Pseudomonas aeruginosa* (MTCC-424), (f) *Pseudomonas aeruginosa* (clinical) and (g) *Salmonella typhimurium* (MTCC-98).

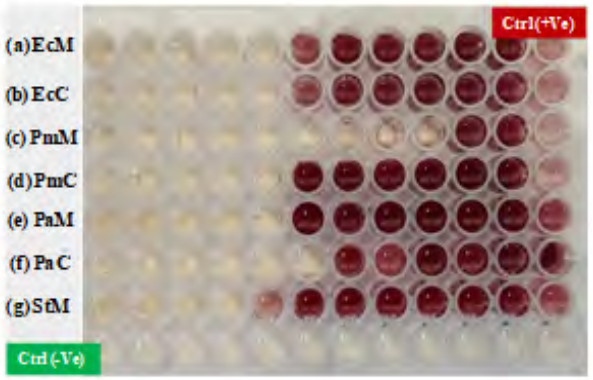


Figure 7. Minimum bactericidal concentration of biosynthesized silver nanoparticles against (a) *Escherichia coli* (Clinical), (b) *Escherichia coli* (MTCC-40), (c) *Proteus mirabilis* (MTCC- 425), (d) *Proteus mirabilis* (clinical), (e) *Pseudomonas aeruginosa* (MTCC-424) (f) *Seudomonas aeruginosa* (clinical) and (g) *Salmonella typhimurium* (MTCC-98).

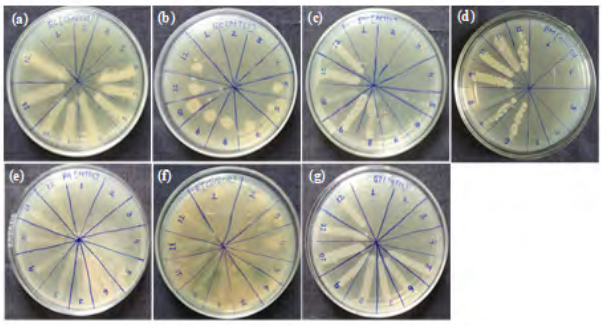


Table.1. Minimum inhibitory concentration (MIC) of biosynthesized silver nanoparticles.

	Name of micro-organisms	MIC
a.	*Escherichia coli* (MTCC- 40)	0.0625 mg/ml
b.	*Escherichia coli* (clinical)	0.0625 mg/ml
c.	*Proteus mirabilis* (MTCC- 425)	0.0312 mg/ml
d.	*Proteus mirabilis* (Clinical)	0.0625 mg/ml
e.	*Pseudomonas aeruginosa* (MTCC-424)	0.0625 mg/ml
f.	*Pseudomonas aeruginosa* (clinical)	0.0312 mg/ml
g.	*Salmonella typhimurium* (MTCC-98)	0.1250 mg/ml

Table. 2. Minimum bactericidal concentration (MBC) of biosynthesized silver nanoparticles.

	Name of micro-organisms	MBC
a.	*Escherichia coli* (MTCC- 40)	0.1250 mg/ml
b.	*Escherichia coli* (clinical)	0.1250 mg/ml
c.	*Proteus mirabilis* (MTCC- 425)	0.1250 mg/ml
d.	*Proteus mirabilis* (Clinical)	0.0625 mg/ml
e.	*Pseudomonas aeruginosa* (MTCC-424)	1.000 mg/ml
f.	*Pseudomonas aeruginosa* (clinical)	>1.000 mg/ml
g.	*Salmonella typhimurium* (MTCC-98)	0.125 mg/ml

CONCLUSION

In the studies mentioned above, the biosynthesized silver nanoparticles (AgNPs) were spherical in shape, nanosized, and demonstrated elevated antimicrobial activity against Gram-negative bacteria. The tolerance range of 2-4 for *Escherichia coli, Salmonella typhimurium*, and *Proteus mirabilis* indicates their bactericidal properties, while a tolerance level of 16 indicates their bacteriostatic effect against *Pseudomonas aeruginosa.*

Author's contributions: RRS and SY planned, designed, and performed the experiments. RRS and AKS prepared the draft and participated in manuscript writing. MKP corrected the draft and finalized the manuscript with RRS. MKP and AKG provided the experimental facilities, analysed, and supervised the work. All authors read and approved the final manuscript before submission.

Declaration of competing interest: There is no conflict of interest regarding the publication of this paper.

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Declaration of generative AI in scientific writing: Generative AI tools such as QuillBot were used solely for language enhancement and paraphrasing; all scientific content, analysis, and conclusions were developed independently by the authors.

Data availability: Data will be made available on request.

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Induced Mutagenesis and Morphological Screening in M2 Generation of Chickpea, *Cicer arietinum* Varieties Vishal and JAKI-9218

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ABSTRACT

The present study investigates the frequency and spectrum of morphological mutations induced by gamma rays, sodium azide (SA), and ethyl methanesulfonate (EMS) in the M2 generation of two chickpea (*Cicer arietinum* L.) varieties, Vishal and JAKI-9218. Seeds of both varieties were treated with each mutagen, and the resulting M2 populations were systematically screened for macromutations. A diverse array of morphological mutants was identified, including tall, dwarf, bushy, prostrate, one-sided branching, narrow-leaved, broad-leaved, open-flowered, early maturing, and chlorophyll-deficient types. The mutation frequency and spectrum varied notably between the two varieties and among the different mutagen treatments, indicating both genotype-dependent mutagenic response and trait-specific mutagenic efficiency. These findings highlight the effectiveness of gamma rays, SA, and EMS in inducing useful genetic variability in chickpea and demonstrate their potential application in crop improvement programs.

KEY WORDS: CHICKPEA, INDUCED MUTATIONS, MORPHOLOGICAL MUTANTS, M2 POPULATION.

INTRODUCTION

Morphological characterization is a foundational tool in plant breeding and genetic research, involving the systematic assessment of observable traits to identify and classify genotypes. It plays a vital role in detecting genetic variation and mutations within crop populations, thereby supporting both taxonomic classification and the improvement of economically important traits. In chickpea (*Cicer arietinum* L.), morphological traits serve as key indicators for distinguishing varieties and understanding the genetic basis of agronomic characteristics. As noted by Singh and Dahiya (1974), morphological analysis also offers valuable insights into phylogeny, illuminating the evolutionary development and functional relevance of specific traits across genotypes.

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Beyond its taxonomic applications, morphological characterization is integral to practical breeding programs. It enables the identification and selection of superior individuals based on desirable traits, facilitates the assessment of genetic diversity, and complements molecular approaches such as marker-assisted selection. In particular, the evaluation of yield-related traits is crucial for developing high-yielding cultivars. At the local level, morphological traits help guide the selection of varieties suited to specific agro-climatic conditions, while globally, they contribute to germplasm conservation, the assessment of genetic diversity, and the development of climate-resilient cultivars—thereby promoting food security and sustainable agriculture.

Induced mutagenesis, employing physical and chemical mutagens, has emerged as an effective strategy for creating genetic variability and novel allelic combinations. Gamma rays, a commonly used physical mutagen, along with chemical mutagens such as ethyl methanesulfonate (EMS) and sodium azide (SA), have demonstrated high efficiency in inducing mutations and generating phenotypic diversity

in a wide range of crops (Borkar and More, 2010 and Koche and Saha 2024).

In this context, the present study was undertaken to induce genetic variability in two chickpea varieties, Vishal and JAKI-9218, through treatment with gamma rays, EMS, and SA. The objective was to generate and characterize morphological mutants in the M2 generation, with the aim of identifying useful variants for potential incorporation into chickpea improvement programs.

MATERIAL AND METHODS

Seeds of two chickpea (*Cicer arietinum* L.) cultivars, Vishal and JAKI-9218, were obtained from the Pulse Research Station, Dr. Panjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola, Maharashtra. Uniform, dry, and healthy seeds with 10–12% moisture content were selected for mutagenic treatments. For physical mutagenesis, seeds were exposed to gamma radiation (Co-60) at doses of 100, 200, 300, and 400 Gy using the gamma irradiation facility at the Central Instrumentation Facility, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

For chemical mutagenesis, seeds were pre-soaked in distilled water for 10 hours and then treated with ethyl methanesulfonate (EMS) and sodium azide (SA) at concentrations of 0.1%, 0.2%, and 0.3% for 4 hours under controlled laboratory conditions. Post-treatment, seeds were thoroughly washed under running tap water for one hour to neutralize residual chemicals. Each treatment consisted of 320 seeds. Treated and control (untreated) seeds were sown in a randomized block design (RBD) with a spacing of 15 cm between plants and 30 cm between rows to raise the M1 generation. Individual M1 plants were harvested separately, and their seeds were used to establish the M2 generation at the experimental field in Jaipur village, Taluka Sengaon, District Hingoli, Maharashtra.

In the M2 generation, plants were systematically screened for morphological mutations. Observations were recorded on five key traits: plant height (cm), growth habit, leaf morphology, branching pattern, and chlorophyll abnormalities. Mutation frequency was calculated, and the collected data were subjected to appropriate statistical analysis to evaluate the significance of induced variation.

RESULTS AND DISCUSSION

The morphological mutants were observed and studied in various traits i.e. plant height mutants, growth habit mutants, leaves variant mutants, flower mutants, Pod size mutants, chlorophyll mutants. After recording the total number of mutants from each dose, their values were reported in the form of their frequency per mutagen.

Table 1.1 Effect of mutagens on the frequency of Morphological mutants in M2 generation of Chickpea variety Vishal

Mutagens & Dose/Conc.	Total Plants observed	PHM	GHM	LVM	FLM	PM	CLM	Freq. (%) of Total Morpho. Mutants
100 Gy	437	0.1163	0.16	0.09	0.18	0.06979	0.18	8.01
200 Gy	428	0.1861	0.18	0.13	0.16	0.11633	0.27	10.75
300 Gy	425	0.2326	0.11	0.13	0.13	0.06979	0.32	10.35
400 Gy	418	0.2559	0.13	0.11	0.23	0.13959	0.37	12.92
S.A.0.1%	436	0.0465	0.06	0.06	0.67	0.06979	0.16	10.78
S.A.0.2%	431	0.1628	0.04	0.06	1.04	0.06979	0.18	15.78
S.A.0.3%	427	0.2093	0.04	0.09	0.93	0.11633	0.25	16.63
EMS 0.1%	435	0.1395	0.11	0.09	0.37	0.09306	0.20	10.11
EMS 0.2%	432	0.2093	0.09	0.13	0.44	0.11633	0.27	12.73
EMS 0.3%	429	0.2559	0.11	0.09	0.44	0.09306	0.30	13.05
Total	4298	1.8147	1.09	1.04	4.63	0.95393	2.56	12.10

Frequency of Morphological Mutants: The frequency of the morphological mutants in the Gamma rays of variety Vishal is reported from range 8.01%, 10.75%, 10.35% and 12.92% at 100 Gy, 200 Gy, 300 Gy and 400 Gy respectively while in variety Jaki it ranges from 5.62%, 7.89%, 9.64% and 10.82% at the same doses respectively. In SA the frequency in Variety Vishal ranges from 10.78%, 15.78% and 16.63% at 0.1%, 0.2% and 0.3% concentration respectively while in Jaki the values are range from 7.32%, 10.75% and 11.71% at .1%, 0.2% and 0.3% concentration respectively. The values of morphological mutant frequency were recorded in EMS treatment reveals that in variety Vishal it ranges between 10.11% to 13.05% at 0.1% to 0.3% concentration, but in variety Jaki the values ranged from 9.81% to 17.85% at

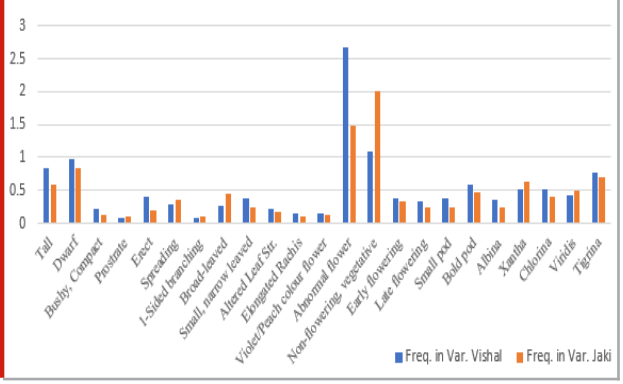
0.1% to 0.3%. The frequency of morphological mutants in both varieties show increasing order as the concentration or doses of treatments increases. The largest values were reported from variety Vishal (12.10%) as compared to variety Jaki (10.64%) (Table 1.1, 1.2).

Table 1.2: Effect of mutagens on the frequency of Morphological mutants in M2 generation of Chickpea variety Jaki.

Mutagens & Dose/Conc.	Total Plants observed	PHM	GHM	LVM	FLM	PM	CLM	Freq. (%) of Total Morpho. Mutants
100 Gy	427	0.0951	0.11	0.06	0.09	0.07140	0.11	5.62
200 Gy	418	0.1189	0.06	0.09	0.16	0.09520	0.23	7.89
300 Gy	415	0.1903	0.06	0.11	0.23	0.07140	0.25	9.64
400 Gy	416	0.2379	0.09	0.13	0.20	0.09520	0.27	10.82
S.A.0.1%	437	0.0475	0.06	0.06	0.39	0.04760	0.11	7.32
S.A.0.2%	428	0.0951	0.06	0.09	0.55	0.04760	0.21	10.75
S.A.0.3%	427	0.0713	0.09	0.06	0.55	0.09520	0.27	11.71
EMS 0.1%	418	0.1189	0.06	0.11	0.34	0.07140	0.23	9.81
EMS 0.2%	407	0.1665	0.09	0.09	0.72	0.09520	0.32	15.48
EMS 0.3%	409	0.2855	0.09	0.06	0.81	0.04760	0.39	17.85
Total	4202	1.4278	0.83	0.93	4.09	0.71394	2.44	10.64

Abbreviations used: PHM: Plant height mutants, GHM: Growth habit mutants, LVM: Leaves variant mutant, FLM: Flower mutant, PM: Pod mutants, CLM: Chlorophyll mutants, TMM: Total morphological mutants.

Figure A: Frequency of Morphological mutants screened from the M2 Population of Mungbean [C. arietinum L., Var- Vijay and Jaki



Plant height mutants: The average plant height in control plants of both the selected varieties is ranges from 35 cm to 40 cm. The plant height mutants were reported at maturity in two chickpea varieties (Vishal and Jaki) when pods were fully developed. The average height of tall mutants in Variety Vishal is observed to be 71 cm to 76 cm. while in Jaki the height ranges from 65 cm to 71 cm. The average height in dwarf plants in variety Vishal was found in between 10.00 cm to 20 cm. while in Jaki it ranges from 08 cm to 18 cm.

A total of 78 plant height mutants were reported from variety Vishal, among these 36 (46.15%) were tall and 42 (53.85%) were dwarf, while in variety Jaki total of 60 plant height mutants were reported, among these 25 (41.67%) were tall and 35 (58.33%) were dwarf (Fig. 1-2).

Growth habit mutants: In M2 generation of Chickpea five types of growth habit mutants were reported from both the selected varieties. Growth habit type mutants include; Bushy, Prostrate, Erect, spreading and one- sided branching.

Frequency of growth habit mutants: The frequency of growth habit mutants in both varieties, Vishal and Jaki, was variable in values. In a variety Vishal of Gamma ray treatments, frequency at 100 Gy to 400 Gy is recorded to be 0.16 %, 0.18%, 0.11% and 0.11% subsequently. In variety Jaki the values were ranges from 0.09%, 0.13%, 0.13% and 0.11% at 100 Gy to 400 Gy doses of Gamma ray, 0.06%, 0.06%,0.09% at 0.1% to 0.3% doses of SA subsequently while 0.09%, 0.13%, 0.09% at 0.1% to 0.3% of EMS doses subsequently (Fig. 3-6).

Leaves variant mutants: Leave Variant mutants also contribute in the spectrum of total morphological mutants which were isolated from M2 Population of Chickpea from both the varieties. The overall frequency of leaves Variant mutants in variety Vishal (1.04%) was observed more in value than variety Jaki (0.93%). A total four types of leaf variants mutants were reported from M2 population of

Chickpea from both the selected varieties i.e. broad leaves mutants, narrow leaves mutants, altered leaf structure mutants and elongated rachis mutants (Fig. 7-10).

Table 1.3: Frequency of different morphological mutants isolated in M2 generation of Chickpea variety Vishal and Jaki.

Mutant Type	Var. Vishal	Freq. in Var. Vishal	Var. Jaki	Freq. in Var. Jaki
Tall	36	0.84	25	0.59
Dwarf	42	0.98	35	0.83
Bushy, Compact	10	0.23	05	0.12
Prostrate	04	0.09	04	0.10
Erect	17	0.40	08	0.19
Spreading	12	0.28	15	0.36
1-Sided branching	04	0.09	04	0.10
Broad-leaved	11	0.26	19	0.45
Small, narrow leaved	16	0.37	10	0.24
Altered Leaf Str.	10	0.23	07	0.17
Elongated Rachis	07	0.16	04	0.10
Violet/Peach colour flower	07	0.16	05	0.12
Abnormal flower	115	2.68	62	1.48
Non-flowering, vegetative	47	1.09	85	2.02
Early flowering	16	0.37	14	0.33
Late flowering	14	0.33	10	0.24
Small pod	16	0.37	10	0.24
Bold pod	25	0.58	20	0.48
Albina	15	0.35	10	0.24
Xantha	22	0.51	27	0.64
Chlorina	22	0.51	17	0.40
Viridis	18	0.42	21	0.50
Tigrina	33	0.77	30	0.71
Total	520	12.10	447	10.64

Flower mutants: Flowers are the reproductive structure of any plant, which affects the overall yield of crops. The changes in the flower structure were observed in both the selected varieties of Chickpea. From the progeny of M2 generation, five kinds of flower mutants were recorded i.e. Flower Colour, Abnormal flower, Non-flowering, Early flower and late flowering mutants. Cumulatively, the frequency of flower mutants was represented from each selected variety of Chickpea. The highest frequency (4.63%) was reported from variety Vishal as compared to variety Jaki (4.09%) (Fig.11-18).

Pod size mutants: Pod size is a remarkable trait that effects on yield of plant. As compared to pods of Control, significant variations occur in the pod size in some mutant plants. Pod size mutants were reported from different doses of both the selected varieties of chickpea. The size of pods corresponded to the size of leaflets. The larger the leaflet size more bolder the pod size whereas smaller the leaflet size in smaller pod size. The frequency of total pod mutants from variety Vishal (0.95393) was higher as compared to Jaki (0.71394) (Fig. 19-24).

Chlorophyll mutants: Chlorophyll mutants are more common in mutational breeding program. Alternation in the pigments may occurs due to change in gene sequence caused by mutations. In Chickpea leaf colour were changed in mutants of different doses. Different kinds of chlorophyll mutants were reported from both selected varieties of chickpea. Isolated Chlorophyll mutants include; Albina, Xantha, Chlorina, Viridis and Tigrina. The frequency of total chlorophyll mutants from variety Vishal was analysed as 2.56% whereas frequency was 2.44% from variety Jaki. In variety Vishal the highest number (16) of chlorophyll mutants were reported from 400 Gy dose and lowest number (7) was reported from S.A. 0.1% dose. Among all identified chlorophyll mutants from every dose, the highest number (33) was reported from Tigrina types while lowest number (15) from Alibina (Fig. 25-29).

Figure 1:

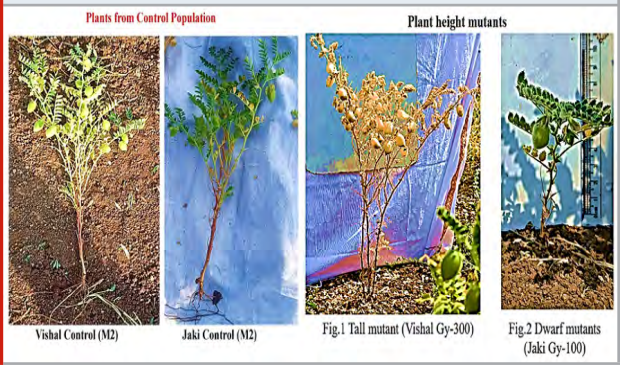
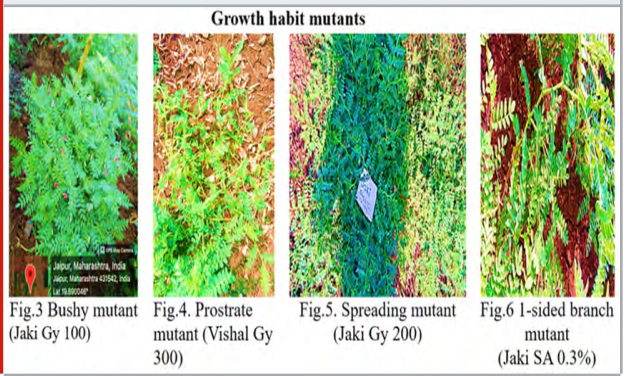


Figure 2:



The present study successfully demonstrated the effectiveness of gamma rays, ethyl methanesulfonate (EMS), and sodium azide (SA) in inducing a broad spectrum of morphological mutations in the M2 generation of chickpea (*Cicer arietinum* L.) cultivars Vishal and JAKI-9218.

Figure 3:

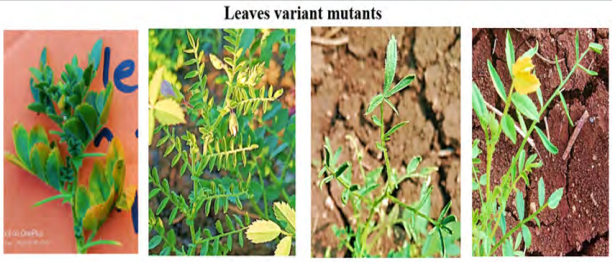


Fig.7. Broad leaves (Jaki EMS 0.1%) | Fig.8 Narrow leaf mutant (Jaki SA 0.3%) | Fig.9 Altered leaf architecture (Vishal EMS 0.3%) | Fig.10.Elongated rachis (Jaki EMS 0.1%)

Figure 4:

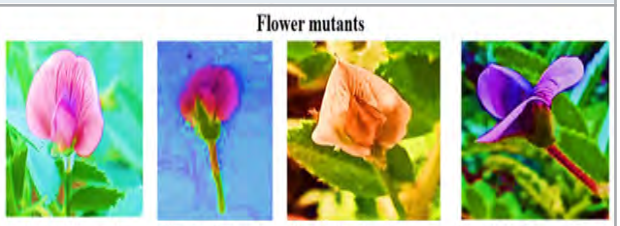


Fig.11 Flower in Vishal Control | Fig.12 Flower in Jaki Control | Fig.13 Peach colour Flower Mutant, Jaki; SA 0.3% | Fig.14 Dark purple colour Flower mutant, Jaki; SA 0.2%

Figure 5:

Fig.15 Abnormal opened flower mutant Jaki EMS 0.3% | Fig.16 Nonflowering/Veg. mutant (Jaki Gy-200) | Fig.17 Early flowering mutant (Vishal Gy-100) | Fig.18 Late flowering mutant (Jaki EMS 0.1%)

Figure 6:

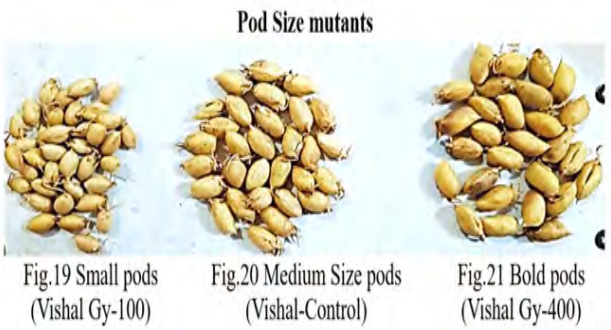


Fig.19 Small pods (Vishal Gy-100) | Fig.20 Medium Size pods (Vishal-Control) | Fig.21 Bold pods (Vishal Gy-400)

The induced variability affected several traits, including plant height, growth habit, leaf morphology, floral characteristics, pod size, and chlorophyll pigmentation—traits of agronomic importance in chickpea improvement.

Figure 7:

Fig.22 Small pods (Jaki Gy-100) | Fig.23 Medium Size pods (Jaki -Control) | Fig.24 Bold pods (Jaki Gy-200)

A clear dose-dependent relationship was observed in most traits, with higher concentrations of mutagens generally resulting in increased mutation frequencies. In cultivar Vishal, the highest frequency of morphological mutations was recorded at 0.3% SA, while in JAKI-9218, the highest mutation frequency was induced by 0.3% EMS. Conversely, the lowest mutation rates were recorded at 100 Gy gamma radiation in both varieties, suggesting that the effectiveness of mutagens is both dose- and genotype-dependent. These findings align with earlier reports of genotypic differences in mutagenic sensitivity (Lal & Mishra, 2006; Khan & Goyal, 2009; Lavanya et al., 2011).

Tall mutants were predominantly observed at higher doses of gamma rays and SA in Vishal, and across 100–300 Gy gamma ray treatments in JAKI-9218. These increases in plant height are likely attributed to enhanced internodal elongation, as reported in previous studies on black gram, lentil, and sesame (Jana, 1963; Sudharani, 1990; Begum et al., 1995). In contrast, dwarf mutants were more frequent under SA and EMS treatments in Vishal and showed a dose-dependent increase under gamma radiation in JAKI-9218. Dwarfism may be due to impaired cell division and elongation caused by mutagen-induced disruptions, consistent with findings by Sonavane (2000) and Dahiya et al. (1984).

Leaf morphological mutants, including broad, narrow, elongated rachis, and altered leaf structures, were observed in both varieties. These anomalies may be the result of chromosomal aberrations or reduced mitotic activity induced by mutagenic stress, as noted by Wani and Anis (2008). Variants in growth habit—such as bushy, prostrate, erect, spreading, and one-sided branching—were detected across treatments, with bushy types being most frequent at the lowest gamma dose (100 Gy). The inconsistent distribution of these mutants suggests polygenic control and complex genetic interactions influencing plant architecture, similar to brachytic mutants reported by Gaur et al. (2008).

Floral mutants, including early and late flowering, non-flowering, and aberrant floral forms, were more prevalent under higher doses of EMS and SA in JAKI-9218. No clear dose-response trend was observed in Vishal, indicating

genotype-specific floral sensitivity. Similar mutagen-induced floral alterations have been documented in legumes (Sonavane, 2000; Kulthe, 2003). Pod size mutations showed variable trends: small pod types were frequent at lower mutagen doses, while bold pod mutants appeared more commonly at higher doses. These observations corroborate findings in urdbean and chickpea by More (2004), Wani and Anis (2008) and Bogawar et al., (2017). Chlorophyll-deficient mutants—such as albina, xantha, chlorina, viridis, and tigrina—exhibited a clear dose-dependent increase across all treatments, with JAKI-9218 recording higher frequencies than Vishal. These mutants, which reflect disruptions in the chlorophyll biosynthesis pathway, are widely recognized as sensitive indicators of mutagenic effects (Sarkar & Kundagrami, 2018; Ahir et al., 2023).

Overall, the results indicate that chemical mutagens, particularly EMS and SA, were more effective than gamma rays in inducing morphological variability. The observed genotypic differences in mutation frequencies and spectra emphasize the need to consider varietal responses in mutation breeding. These findings reinforce the utility of induced mutagenesis as a valuable tool for generating genetic variability, which can be harnessed for chickpea improvement through the selection of desirable mutants in subsequent generations.

CONCLUSION

This study demonstrated that induced mutagenesis using gamma rays, EMS, and sodium azide effectively generated a broad spectrum of morphological mutations in chickpea (*Cicer arietinum* L.). Significant variability was observed in key agronomic traits, including plant height, growth habit, leaf morphology, floral structure, pod size, and chlorophyll pigmentation. Among the two cultivars, JAKI-9218 exhibited greater sensitivity to mutagenic treatments than Vishal, with the highest mutation frequencies recorded at 0.3% EMS and 0.3% SA. While gamma irradiation was comparatively less effective, it still produced useful mutations at moderate doses. The morphological mutants obtained—especially those influencing plant architecture and yield-related characteristics—hold considerable potential for use in chickpea improvement programs. Further evaluation and selection of promising lines could facilitate the development of superior chickpea varieties with improved agronomic performance, genetic diversity, and adaptability to diverse agro-climatic conditions.

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Conflict of Interest: The authors declared no conflicts of interest with this research work.

Data Availability: All data will be available with the corresponding author on reasonable request.

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Assessing Quality of Life in Lower Limb Amputees with Prosthetic Devices: A Systematic Review

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ABSTRACT

Lower limb amputations impact mobility, health, social independence, psychological well-being, and economic stability. Accidents are the leading cause in developing countries, like India. affecting amputees' quality of life. However, pain and dissatisfaction remain challenges. This systematic review aimed to explore the impact of current trends and various factors on patient satisfaction with lower limb prostheses, and their influence on health-related quality of life, drawing insights from qualitative literature. We searched the databases, including Web of Science, Saudi Digital Library (EBSCO), Science Direct and Google Scholar for systematic reviews, involving Quality of Life, and functional instruments for lower limb amputees with prostheses. Published articles from 2008 to 2023 were selected. Participant characteristics, inclusion criteria, outcome measures, study design, and results were explicated. Thirteen articles, published between 2008 and 2023, met the inclusion criteria. Factors impacting quality of life include cognitive ability, the cause of the amputation, amputation level, early prosthesis use, employment status, and social and psychological aspects. Additional factors include age, gender, marital status, education, income, and time since amputation.This study explores the relationship between lower limb amputation and quality of life, focusing on factors like amputation level, etiology, physical activity, social and psychological aspects, cognitive ability, pain, comorbidities, discomfort, accessible devices, work status, income, education, and living region. Prosthesis users experience superior QOL.

KEY WORDS: HEALTH, PROSTHESIS, AMPUTATION, LOWER LIMB, QUALITY OF LIFE.

INTRODUCTION

Lower limb amputations cause inconveniences in walking and affect other daily activities along with psychological and social challenges (Day, Wadey and Strike, 2019). Limb amputation significantly impacts a person's quality of life, affecting mobility, physical health, social independence, psychological status, and economic status (Sinha, van den Heuvel and Arokiasamy, 2011). Amputations may be due to congenital, pathological, or traumatic events may be the cause of an amputation(Havard, Trauma and 2025, no date).

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A comprehensive review of studies is required to understand amputees' quality of life, improve rehabilitation and enhance healthcare professionals' knowledge.A study published in 2021 revealed that globally, 57.7 million individuals were grappling with limb amputation stemming from traumatic incidents. Among the primary traumatic causes identified were falls (36.2%), road injuries (15.7%), other transportation-related accidents (11.2%), and mechanical forces (10.4%) (McDonald *et al.*, 2021). In India, the overall rate of disability is 4.52%, with locomotor disabilities accounting for 44.70% of cases (Mohanty, Mohanty and Sabut, 2020; Pattnaik *et al.*, 2023).

Accidents are the primary cause of most lower limb amputations in India. Additional investigations from

Chennai and Kolkata supports this fact (Pooja and Sangeeta, 2013a; Mukundan *et al*., 2020). The quality of life is greatly impacted by the role of prostheses; Characteristics with a higher impact include a healthy stump, immediate prosthesis fitting, alignment and gait training, high-quality prostheses, amputee satisfaction, income source, and social and family support(Rafi, 2020). Factors that adversely impact quality of life include older age, duration of time after amputation, lack of family and social support, short amputation level, maximum functional loss, job satisfaction, family education, and the presence of disease (Sinha, van den Heuvel and Arokiasamy, 2011; Davie-Smith *et al*., 2017; Zaheer *et al*., 2020; Fatima, 2023).

Amputees can walk and perform daily activities with the help of a suitable prosthetic device, boosting their activity, independence, and happiness (Sindwani *et al*., 2023). The social reintegration of the patient may benefit even from an aesthetically pleasing prosthetic device. Numerous studies have investigated functional performance, health status, mobility level, and predictive factors in lower limb amputation. However, these findings are rarely associated with prosthetic satisfaction or health-related factors of life(Brunelli *et al*., 2020; Roberts *et al*., 2021; Dade Matthews, 2022; Norvell *et al*., 2024).

Notably, pain, particularly low back pain or phantom pain, significantly impacts a person's poor quality of life in addition to their physical disability, (Polat *et al*., 2021). In India, the number of traumatic lower limb amputees is higher than those from other pathological conditions, (Kumar *et al*., 2020).Younger, healthier traumatic injury patients generally recover faster and adapt to prosthetics more easily, although they may struggle with the psychological impact of sudden limb loss(Sanders *et al*., 2020). With proper support, they often respond well to rehabilitation. In contrast, Patients with peripheral vascular injuries often experience slower healing and more complications during prosthesis fitting and recovery due to poorer circulation and comorbidities, (Day *et al*., 2023). Older age and other health conditions can further delay rehabilitation and prosthetic adaptation, emphasizing the need for comprehensive management for successful recovery(Knight, Dearth and Hendershot, 2021). Furthermore, early-life traumatic amputation is often linked to improved quality of life compared to amputation due to other pathological conditions (Vieira *et al*., 2024).

Strategies to select article between 2008-2023 aimed to understand advancements in prosthesis fitting in lower limb amputees that may affect health related quality of life. "Despite an extensive search conducted over the past 16 years, no systematic review studies addressing quality of life for lower limbs amputees in the context of India and the Asian region were found. "In recent years, the landscape of lower limb prosthetic services in India has witnessed a significant shift with numerous private organizations establishing centers across various regions. These centers have played pivotal roles in technology transfer and innovation. Consequently, both private and government entities have actively engaged in the fitting and provision of lower limb prostheses. This diversification has empowered patients, offering them choices beyond government-run facilities, and free camps(Pal, 2020). Improved financial conditions and income diversification enable individuals to invest in health and quality of life through private healthcare options, fostering employment and integrating prosthetic technology into daily routines, creating a promising avenue in lower limb prosthetics (Baru and Nundy, 2020; Poonekar and Gupta, 2022; Neelakantan and Kulkarni, 2023).

METHODOLOGY

We adhered to the Moher et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher *et al*., 2009) We screened qualitative studies whose main objective was to use reliable and valid tools, techniques, or systems that might be used to evaluate how prosthetic fitting affected mobility, physical health, and quality of life. Most included studies are sourced from Asian publications, with a limited representation from other geographical regions.

Search Strategy- We searched the following databases: Web of Science, Saudi Digital Library (EBSCO), Science Direct and Google Scholar for studies including QOL for lower limb amputees with prostheses. Key phrases including "quality of life," "lower limb," "prosthesis," "amputation," and "health" were used to search for relevant information. The database was searched from its creation until 2023, and only English-language journal articles, review papers, and clinical research papers were included. The search was performed by first author (RK) on 24 February 2024.

Table 1. Search strategy for databases.

Search Database	Domain Code	Search terms
Web of Science	Title	lower limb* AND amputation* AND quality of life*
SDL(EBSCO)	Title	lower limb amputee* AND quality of life AND PT academic journal*
Science Direct	Title, abstract, keywords	lower limb* AND amputation* AND quality of life*
Google scholar	All in title	quality of life* OR lower limb* OR amputation*

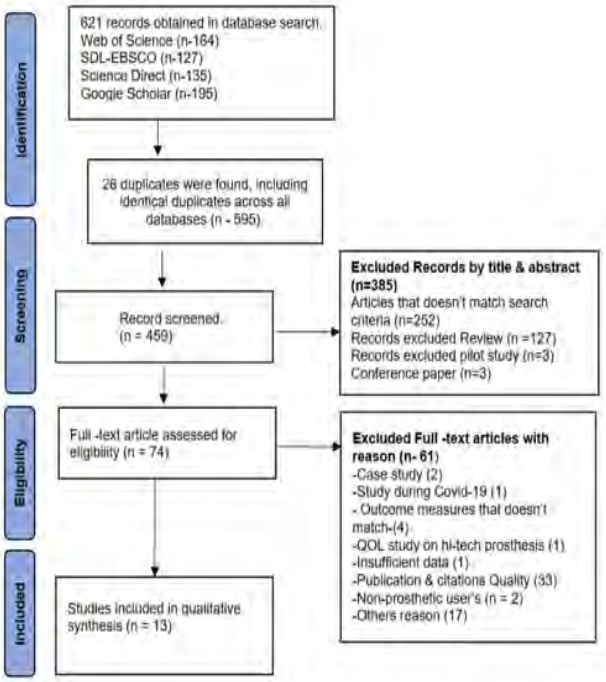
Inclusion and Exclusion: The study includes individuals with unilateral or bilateral transtibial, knee, transfemoral, or hip joint amputations, as well as prosthesis users, and their quality-of-life issues. We included studies using various research methods including randomized controlled trials, quasi-randomized controlled trials, controlled trials,

cohort studies, or cross-sectional studies. Qualitative Studies published between 2008 and 2023 were eligible for inclusion.

Participants should have undergone lower limb amputations due to congenital, traumatic, vascular, or tumor-related causes, in consultation with professionals and caregivers. Studies that do not focus on the use of prostheses were excluded. Articles published before 2008 were not considered. Publications in languages other than English were excluded. Studies involving participants below the age of 18 were excluded. Articles including case reports, reviews, editorial opinions, testimonials, biographies/ interviews, books, or discussions unrelated to the quality of life in amputees with of their lower limbs were excluded.

Selection Criteria: All identified studies published between 2008 and 2023 were imported using CSV and BibTeX formats, and subsequently transferred to an Excel (2016) sheet for organization. which were then compiled into the Excel sheet. Duplicate entries were removed, and the remaining data underwent thorough scrutiny based on predefined inclusion and exclusion criteria. Figure-1 illustrates the selection procedure. Investigators R.K independently screened the relevant research articles from Web of Science, Saudi Digital Library (EBSCO), Science Direct and Google Scholar databases. Author -3 (R.K) reviewed exclusion and inclusion criteria, electronic article relevancy, abstract suggestions and instructed to follow PRISMA pattern for the review study. Senior author-2 (AJG) verified the result and finalized the methodological quality of the study. Publications that addressed health-related quality of life were excluded if any of the inclusion criteria were not satisfied.

Figure: - 1. Flow chart PRISMA assessment.



Data Extraction: Data were retrieved by the first author (RK) regarding the author, year of publication, country of publication, publication in a journal and the study characteristics (including participant number, mean age, gender, marital status, education, amputation level, amputation cause, and prosthesis use), inclusion/exclusion, outcome measures, study design, results and conclusion based on Butler *et al.'s* research design (Butler *et al*., 2000) The initial search yielded 621 research articles from various databases, from which we extracted 13 records.

Study Quality: We followed the advice given by Butler *et al.* (2016) for qualitative studies, using the Critical Appraisal Skills Program (CASP, 2018). A 10-point scale was used for critical evaluation in a quality study we did. Each CASP item received a score of "yes" (1 point), "unsure" (0.5 points), or "no" (0 points). High-quality papers scored between 9 and 10, while moderate quality papers scored 7.5–9. Low quality papers less than 7.5 or less than 6 were excluded. The careful evaluation was done by the first author (RK). Due to the standards suggested by Butler et al. (2016), studies were not ruled out ("Critical Appraisal Skills Programme. CASP," no date; Butler *et al*., 2000).

RESULTS AND DISCUSSION

A total of 621 studies were found using four databases: Google Scholar (n =195), SDL-EBSCO (n =127), Science Direct (n =135), and Web of Science (n =164). After reviewing the title and abstract 385 studies were eliminated for not meeting the inclusion criteria, 13 studies were removed as duplicates, and 13 studies were identical duplicates across all four databases. Sixty-one full-text articles were rejected for reasons during the second screening, leaving 74 full-text articles that were still accessed for eligibility. Thirteen research studies are included in this evaluation figure 1. The studies were conducted in India (n = 6), Malaysia (n = 1), Saudi Arabia (n = 1), Serbia (n = 1), the United Kingdom (n = 1), the Netherlands (n = 1), Finland (n = 1), and Brazil (n = 1). The studies included were published between 2008 and 2023.

According to the qualitative assessment result checklist for critical appraisal, all the studies scored between 8 and 10 points on the CASP scales. Ten were of high quality, and three received a moderate score ("Critical Appraisal Skills Programme. CASP," no date)

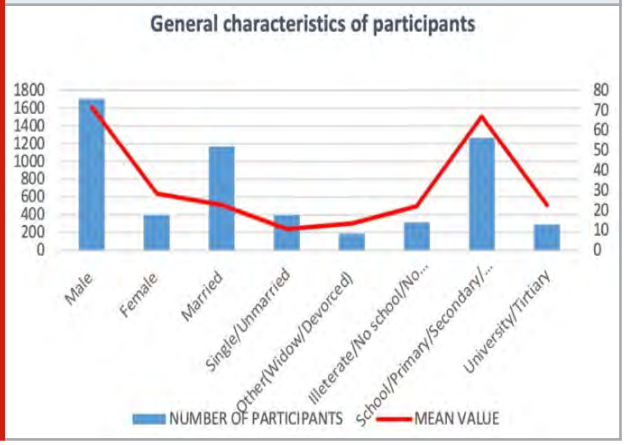
The studies' heterogeneity led to data synthesis completion through descriptive analysis. There were 2108 participants in the study, with a mean age of 50.55. Of these, 400 (18.95%) were female and 1708 (81.05%) were male. Among the participants, 1169 (66.77%) were married, 397 (22.67%) were single or unmarried, and 185 (10.56%) were divorced or widowed. Only Remes et al. discussed the marital status categories of unmarried, divorced, and widowed together (Remes *et al.*, 2010). Fortington, Knežević, Magnusson, Deepak, and Priyadharshan et al. did not specify the participants marital status (Fortington et al., 2013; Knežević et al., 2015; Magnusson et al., 2019; Priyadharshan et al., 2022; Deepak *et al.*, 2023).

315 individuals (16.71%) reported being illiterate, while 1269 participants (67.65%) reported having received schooling, vocational education, or primary and secondary education. Additionally, 292 participants (15.56%) reported having obtained university or tertiary-level education. It's worth noting that Fortington et al., Knežević *et al*., and Priyadharshan et al. did not provide education reporting in their studies (Fortington et al., 2013; Knežević *et al*., 2015; Priyadharshan *et al*., 2022, 2022)

Table- 2 CASP Quality assessment

Study Author, Year [References]	Clear statement of the aim	Qualitative methodology appropriate	Research design appropriate	Recruitment strategy appropriate	Data collection Addressed research issue.	Relationship between researcher and participants adequately considered	Ethical issues taken into consideration	Data analysis sufficiently rigorous	Clear statement of findings	Valuable research Score	Score
Deans *et al.*(2008) [17]	Yes	Yes	Yes	No	Yes	Yes	Can't Tell	Yes	Yes	Yes	8.5
Remes *et al.* (2010) [18]	Yes	Yes	Yes	Yes	Yes	Can't Tell	Yes	Yes	Yes	Yes	9.5
Sinha *et al.*,(2011) [19]	Yes	Yes	Yes	Yes	Yes	Yes	Can't Tell	Yes	Yes	Yes	9.5
Fortington *et al.*, (2013) [20]	Yes	Yes	Yes	Yes	Can't Tell	Yes	Yes	Yes	Yes	Can't Tell	09
Sinha *et al.* (2014) [21]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10
Sinha *et al* (2014) [22]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10
Knežević *et al.*, (2015), [23]	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	10
Razak *et al.*, (2016), [24]	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	8
Magnusson *et al.*, (2019) [25]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10
Matos *et al.*, (2019), [26]	Yes	Yes	Yes	Yes	Can't Tell	Yes	Yes	Yes	Yes	Yes	9.5
Alessa *et al.*, (2022), [27]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	10
Deepak *et al.*, (2023) [28]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9.5
Priyadharshan *et al.* [29]	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	8

Figure-2- General characteristics of participants

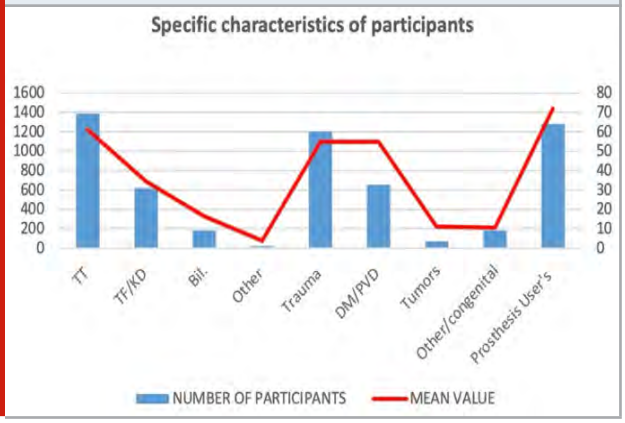


Traumatic injury accounted for 1202 (62.99%) of the participant causes; diabetes mellitus, including vascular disease, accounted for 655 (34.32%); tumors accounted for 70 (3.66%); and congenital conditions accounted for 181(9.48%). While Razak et al. collectively discuss vascular disease and infection in 24 (57.2%) cases, Alessa et al. collectively reported on vascular cases, diabetes, and infection in 96 (40.2%) instances (Razak *et al*., 2016; Alessa *et al*., 2022).

In total number of participants with various amputation levels was 1385 (65.7%) for transtibial amputations, 621 (29.46%) for transfemoral with knee disarticulation, 81 (3.85%) for bilateral amputations, and 21 (0.99%) for others. Among these, 1283 participants (or 60.83.2% of the total) used prostheses. Studies by Fortington et al. indicate that most participants are prosthesis users, while Deans et al., Sinha et al. in 2014 study and Knežević et al. mention prosthesis users in their inclusion criteria (Deans, McFadyen and Rowe, 2008; Sinha, Van Den Heuvel and Arokiasamy, 2011; Fortington et al., 2013; Sinha et al., 2014; Knežević et al., 2015).

The average study looks at adult men and women aged 18

Figure-3- Specific characteristics of participants



and up who have had a lower limb amputated trans-tibial or trans-femoral, or who had their limb amputated because of diabetes, vascular disease, or an infection, and have been in rehabilitation for at least 6 months. People with severe psychopathology, end-stage cancer, left-side hemiparesis, vision impairment, or other psychiatric disorders were excluded in several studies. Additionally, individuals with clinical dementia, an ipsilateral amputation history, difficulty reading or writing Dutch, and recall bias-related selection more than five days after the amputation were excluded by Fortington et al. Magnusson *et al.* excluded from their study protrauma, psychiatric illness, patients over the age of eighteen who had lower limb amputations for more than six months, individuals with amputations, post-amputations, refusal to participate, and hearing, speech, or visual function disorders. They also excluded organization staff and students who used prosthetics or orthotics (Fortington *et al*., 2013; Magnusson *et al.*, 2019)

Most frequently employed outcome measure across the studies reviewed was the MOS SF-36, which encompasses variations such as RAND-36 and SF-12, was utilized a total of seven times Following closely, the PEQ measure was employed in one instance (Harness, Related and 2001, no date; Hays and Morales, no date; Ware, 2000; Condie *et al*., 2006). TAPES and its revised version, TAPES-R, were each utilized six times, respectively. Furthermore, the WHOQOL-Brief was utilized in five instances among the studies reviewed [38]. Remes *et al.* also used self-reported life satisfaction scores, the 6-item Brief Social Support Questionnaire, and the Geriatric Depression Scale. Alessa *et al*. utilized the DASS (Depression, Anxiety, and Stress Scale), 12-item ZBI (Zarit Burden Interview) (Remes *et al.*, 2010) (Deans, McFadyen and Rowe, 2008).

Most of the research talk on participant characteristics such the level and aetiology of amputation. Less often brought up topics are marital status and level of education. Deans *et a*l. have out that social and psychological aspects are critical for improved quality of life, with decreased physical activity being a worry. According to Remes et al., pain and comorbidities are associated with lower QOL, although cognitive ability is positively correlated with higher QOL (Remes et al., 2010). According to Sinha et al. in 2011, prosthesis use, comorbidities, and discomfort relate to lower QOL, but employment status and assistive devices are linked to higher QOL (Sinha, Van Den Heuvel and Arokiasamy, 2011) Sinha et al. in 2014 found that QOL is influenced by employment, prosthesis use, and gender, and that unemployment is linked to poorer social adjustment and QOL (Sinha et al., 2014) (Sinha, Van Den Heuvel and Arokiasamy, 2014) Fortington et al. state that pain, vitality, physical function, social function, and perceived health are the primary elements of an enhanced quality of life [20].

According to Knežević et al., compared to lower amputation levels, transtibial amputations are more functional and have improved health related QOL [23]. Razak et al. discover that while physical domains lead to a lower quality of life, emotional support and psychological components have a stronger effect on QOL (Razak et al., 2016). Magnusson *et al.* highlight the importance of living area, education level, and income in determining quality of life (QOL), noting that physical handicap lowers QOL (Magnusson *et al*., 2019). Matos *et al*. found that prosthesis users had a higher quality of life than non-users, and that major factors contributing to increased QOL were pain, prosthesis adaptation, and psychological well-being (Matos, Naves and de ARAUJO, 2020). Priyadharshan *et al*. discover that variables like age, time since amputation, and prosthesis use contribute to improved QOL (Priyadharshan *et al*., 2022). Deepak *et al.* contend that early prosthesis fitting, and psychological counselling can significantly improve QOL, with delays exacerbating physical burdens and diminishing QOL (Deepak *et al.*, 2023).

This systematic review offers a comprehensive analysis of the demographic and clinical characteristics influencing the quality of life (QoL) among lower limb amputees across diverse geographical regions. The studies underscore the global burden of lower limb amputations and the multifaceted challenges faced by amputees. Notably, the concentration of studies in India reflects regional disparities in research focus and the prevalence of lower limb amputations due to trauma and vascular diseases.

Demographic Characteristics

Age and Gender: The mean age of participants varied widely, highlighting that lower limb amputations affect a broad age spectrum. Predominantly male participants (81.05%) across studies point to gender-specific risks and exposures, particularly to trauma-related injuries. This gender distribution suggests a need for gender-sensitive rehabilitation programs, as men might face different challenges compared to women in adjusting to life post-amputation.

Marital Status: Married individuals consistently reported better QoL compared to their single, divorced, or widowed counterparts. This finding underscores the critical role of social support systems, particularly spousal support, in enhancing psychological well-being and facilitating rehabilitation. The lack of detailed marital status information in some studies suggests that future research should include more comprehensive demographic data to fully understand

the impact of social relationships on QoL (Fortington *et al*., 2013; Knežević *et al*., 2015; Magnusson *et al.*, 2019; Priyadharshan *et al.,* 2022; Deepak *et al*., 2023).

Educational Background: Higher educational levels were associated with better QoL, likely due to improved access to healthcare resources, better understanding of rehabilitation processes, and enhanced socioeconomic status. This relationship indicates the importance of educational interventions in rehabilitation programs, helping amputees leverage their knowledge and skills to navigate post-amputation life more effectively.

Clinical Characteristics: Causes and Levels of Amputation Etiology: The primary causes of amputation included trauma, diabetes, and peripheral vascular disease (PVD), with trauma being the most prevalent. This reflects the higher incidence of accidents and injuries in developing regions, particularly in India(Pooja and Sangeeta, 2013b). Understanding the aetiology is crucial for developing targeted prevention strategies and tailored rehabilitation programs that address the specific needs of different patient groups (Kumar and Kumar Yadav, 2022; Dean, 2009).

Amputation Levels: Transtibial amputations (below-knee) were more common than transfemoral amputations (above-knee). This distinction is significant because transtibial amputees generally experience better functional outcomes, including greater mobility and ease of prosthetic use. These findings highlight the importance of tailoring rehabilitation programs to the specific amputation level to maximize functional recovery and QoL.

Prosthetic Use and Quality of Life: Prosthetic Utilization Approximately 60.83% of participants used prosthetic devices, indicating their critical role in enhancing mobility and overall QoL. However, a significant proportion of amputees did not use prosthetics due to issues such as stump pain, mechanical problems, and psychological barriers. This underscores the need for improving prosthetic technology and addressing psychological factors to enhance prosthetic adoption and effectiveness.

Psychosocial Factors: Emotional support, social integration, and psychological counselling emerged as vital components for successful rehabilitation. Studies consistently highlighted the impact of psychosocial factors, such as depression, anxiety, and phantom limb pain, on QoL. Addressing these issues through comprehensive rehabilitation programs that include psychological support can significantly improve outcomes for amputees(Marques, Journal and 2025, no date).

Assessment Tools: The use of various QoL assessment tools, such as the SF-36, WHOQOL-BREF, and TAPES, reflects the multidimensional nature of QoL among amputees. These tools measure physical, psychological, and social domains, providing a holistic view of the impact of amputation on patients. The widespread use of these validated tools underscores their reliability and importance in assessing rehabilitation outcomes (Stanciu *et al.*, no date; Balk *et al*., 2019)

Key Findings from Individual Studies: Deans et al. (2008) emphasized the importance of social relationships and psychological well-being, finding strong correlations between social elements and QoL scores, underscoring the role of social support in rehabilitation (Deans, McFadyen and Rowe, 2008). Remes *et al*. (2010) identified cognitive ability and proper management of pain and comorbidities as crucial for maintaining functional ability and QoL, and linked institutionalization to higher depressive symptoms, suggesting the need for community-based support systems (Remes *et al*., 2010). Sinha *et al*. (2011) found that employment status and the use of assistive devices were key determinants of QoL, with factors such as prosthesis use, comorbidities, and pain associated with lower QoL, highlighting the multifaceted challenges faced by amputees (Sinha, Van Den Heuvel and Arokiasamy, 2011).

Fortington *et al*. (2013) reported significant improvements in QoL over time, particularly within the first six months post-amputation, though physical function remained below population norms, indicating the need for ongoing support and interventions (Fortington *et al*., 2013). Matos et al. (2019) demonstrated that better QoL was predicted by male gender, time since amputation, and below-knee amputations, with pain, prosthesis adaptation, and psychosocial well-being being significant factors influencing QoL(Matos, Naves and de de Araujo 2020).

Implications for Clinical Practice, Rehabilitation Programs: Effective rehabilitation programs must address both physical and psychological aspects of recovery. Ensuring access to high-quality prosthetic devices, proper fitting, and alignment is crucial. Additionally, integrating psychological counselling and social support services can significantly enhance QoL outcomes for amputees (Shehata *et al.*, 2025).

Educational Interventions: Increasing awareness and education about the importance of prosthetic use and psychological support among amputees and their families can improve rehabilitation outcomes. Tailored educational programs can help patients understand and manage the challenges associated with lower limb amputation.

Policy and Healthcare Services: Policymakers should focus on improving access to prosthetic devices and rehabilitation services, particularly in regions with high rates of trauma-related amputations. Developing comprehensive healthcare policies that address the physical, psychological, and social needs of amputees can enhance their overall well-being (Asano *et al.*, 2008; Baars *et al*., 2018).

Future Research Directions: Longitudinal Studies: There is a need for more longitudinal studies to track changes in QoL over time and understand the long-term impacts of amputation and prosthetic use. Such studies would provide deeper insights into the rehabilitation process and the evolving needs of amputees.

Regional and Cultural Diversity: Expanding research to include diverse geographical and cultural contexts can help identify unique challenges and best practices globally. This

approach would ensure that rehabilitation strategies are inclusive and tailored to the needs of amputees worldwide. Advanced Prosthetic Technologies: Research into advanced prosthetic technologies and their impact on QoL can provide valuable information for improving prosthetic design and functionality. Innovations in prosthetic technology can significantly enhance mobility and overall QoL for amputees.

Table 3 Part A

Details of Research Done	Characteristics of participants – Number (N %) -Age (Mean) - Gender -Marital status -Education -Amputation level -Amputation cause -prosthetic user's	Inclusion and Exclusion	Outcome measures	Study design	Result	Conclusion
Deans et al. (June 2008), UK/ Prosthetics and orthotics international [17]	N- 25 Age (mean)- 66 Gender Male-20 (80%) & Female-5 (20%) Marital status Married -17 (85%) Widowed-3 (15%) Education Secondary -14 (70%) Tertiary- 6 (30%) Amputation level- TT- 22 (89%) & TF-3 (12%) Amputation cause- PVD Prosthesis User's- 25 (100%)	Inclusion- Adult men and women who underwent unilateral trans -tibial or trans -femoral lower-limb amputation and were able to wear and use a prosthesis were included in the study. The top age limit was left unspecified at eighteen.	TAPES, WHOQOL-Brief	cross-sectional study	• Strong correlation between social elements in questionnaires. • Significant relationships between scores in Psychological and Social domains • Statistical correlation between TAPES subscales and WHOQOL-Bref domains. • No relationship between Athletic subscale of TAPES and WHOQOL-Bref domains.	The study's finding that there is less of a correlation between physical activity and amputees' quality of life highlights the significance of their relationships with peers, family, and friends.
Remes et al. (October 2010), Finland/ Prosthetics & Orthotics international [18]	N- 59 Age (Mean)- 75.17 year Gender Male- 28 (47%) Female- 31(53% Marital status	Exclusion- The individuals were excluded due to their inability to respond to all items	RAND-36 Health-Related QoL instrument, Physical Functioning-	Cros-sectional study.	• Depression and anxiety common up to two years post-amputation. • Cognitive ability crucially	Institutionalization is linked to depressive symptoms, while home -dwelling amputees have generally

Table Continued

	Married- 21 (36%) Widowed, divorced, unmarried- 38 (64%) Education- No vocational education -35 (59%) Vocational school or learning at work- 20 (34%) College/ university- 4 (7%) Amputation level- TT- 13 (22%) TF- 28 (48%) Bil.-18 (30%) Amputation cause- PVD Prosthesis user's- 25(100%)	due to vision impairment, end-state cancer, left-side hemiparesis, or severe psychopathology.	and General Health subscales, Geriatric Depression Scale, 6 -item Brief Social Support, Questionnaire, and Self -reported Life Satisfaction score.		affects amputated patients' quality of life. • Proper management of pain and comorbidity may mitigate depressive symptoms. • Feelings of ' perceived control' crucial for maintaining functional ability. • Assessment of depression in elderly crucial as it diminishes quality of life.	good QoL scores. Rehabilitation should integrate QoL assessment with physical disability assessment.
Sinha et al., (March 2011), India/ Prosthetics & Orthotics international [19]	N- 605 Age (Mean)- 43.7 Gender Male -530 (88%) Female -75 (12%) Marital status Married - 436 (72%). Single -117 (19%). Others - 52 (9%) Education- No schooling/missing - 119 (19%) N- 605 Age (Mean)- 43.7 Gender Male -530 (88%) Female -75 (12%) Marital status Married - 436 (72%). Single -117 (19%). Others - 52 (9%) Education- No schooling/missing - 119 (19%) High school- 428 (71%) University- 57 (9%) Amputation level TT - 410 (68%) TF/TK- 151 (25%) Bil. - 29 (5%) Other 15 (2%) Amputation cause Trauma -381 (63%)	Exclusion- The individual may experience a range of psychological disturbances, including a lack of participation , hearing or speech impairment, mental incapacity, or the loss of a family member.	MOS SF-36	cross-sectional study.	• Lower limb amputees had worse QoL compared to the general population. • Employment status and use of assistive devices were key determinants of QoL • Factors like prosthesis use, comorbidities, phantom-limb pain, and stump pain affected QoL.	The study found that lower limb amputees have worse quality of life compared to the general population, with employment status and use of assistive devices playing key roles.

Table Continued

	Diabetes/vascular -135 (22%) Others 88 -(14%) Missing 1 (1%) Prosthesis user's- 66(10.93%).					
Fortington et al., (June 2013), Netherlands/ Journal of rehabilitation and medicine [20]	N- 82 (77%) Age (Mean)- 67.8 year Gender Male- 55 (67%) Female- 27 (33%) Marital status- N/S Education- N/S Amputation level TT -52 (63%) TF/TK- 30(37%) Amputation cause- PVD Prosthesis user' s- 82(100%).	The study included individuals aged 18 and above who had a primary lower limb amputation due to vascular disease, infection, or diabetes. The study excluded individuals with previous ipsilateral amputation, difficulty reading /writing Dutch, clinical dementia, or those recruited over 5 days after the amputation due to recall bias.	Dutch version RAND-36	longitudinal study.	• The quality of life significantly improved in five of the seven domains. • The level of physical function stayed below the population standard. • Age groups may see varying effects in various domains.	In five out of seven domains, the study indicated a significant increase in quality of life; the majority of these improvements happened within the first six months, while physical function remained below population norms.
Sinha et al. (April 2014), India/ Prosthetics & Orthotics international [21]	N- 368 Age- (Mean)- 43.13 year Gender Male- 324 (88%) Female- 44 (12%) Marital status Married -264 (72%). Single -70 (19%). Others - 34 (9%) Education No formal education /missing - 65 (17.7%) Primary/ secondary education - 260 (62%) Tertiary education- 43 (20.3%) Amputation level TT- 281 (76.3%) TF/KD- 87 (23.7%) Amputation cause Trauma 280 (76.1%) Diabetes/	Inclusion- In considering the study's objective, a cross-section of unilateral and non-congenital amputees who were wearing prosthetic limbs was taken into consideration. Exclusion-The study excluded participants due to non-participation, hearing or speech impairment, mental incapacities, and limb fitting and gait training for amputees.	PEQ, TAPES	Cross-sectional study.	• Factors influencing adjustments: age, employment, daily prosthesis use, assistive device use. • Functional satisfaction linked to prosthesis use, phantom pain , employment, and gender. • Unemployed amputees less socially adjusted and restricted in activities.	Amputees who are not limited in their activities except for sports and who are generally content with how their prosthesis functions are also considered to be somewhat psychosocially adjusted. Age, occupation, amputation level, co-morbidity, gender, everyday prosthesis use, and assistive device use all affect modifications.

Table Continued

	vascular 59(16%) Cancer- 14 (3.8%) Others 15 (3.9%) Prosthesis user' s –368 (100%)					
Knežević et al., (April 2015), Serbia/ Medicinski pregled [23]	N- 28 Age (Mean)- 65.36 Gender Male- 21(75%) Female- 7 (25%) Marital status- N/S Education- N/S Amputation level- TT-11 (39%) TF- 17 (61%) Amputation cause- DM/Vascular- All Prosthesis user's – 28 (100%)	Inclusion-The study involved patients with lower extremituies walking ability, and at least 6 months of rehabilitation treatment. Control group included lower extremity presence, independent walking, age and gender homogeneity, and two chronic diseases.	RAND- 36	Cross-sectional Study.	• Patients with lower extremity amputations have reduced quality of life. • Patients with transtibial amputations are more functional and have better health. • SF-36 questionnaire results show differences in physical function and general health.	Lower extremity amputees, regardless of gender, exhibit numerous limitations compared to the control group, while those with lower amputee levels have higher physical functioning.
Razak et al., (June 2016), Malaysia/ Procedia - Social and Behavioural Sciences [24].	N- 43 Age (Mean)- N/S Gender Male 24 (55.8%) Female 19 (44.2%) Marital Status- Married 33 (76.7%) Unmarried 8 (18.6%) Divorced 2 (4.7%) Education No Education 2 (4.7%) Primary/ Secondary - 35(81.4%) Tertiary 6 (14.0%) Amputation level Transtibial 23 (54.8%) Transfemoral 16 (38.1%) Bilateral 6 (14.0%) Amputation cause- Trauma 14 (33.3%) Infection /Vascular Disease - 24 (57.2%) Congenital 5 (9.5%) Prosthesis user's -25 (58.1%)	The study included adult men and women with unilateral or bilateral lower limb amputations who were undergoing post-amputation rehabilitation programs.	WHOQOL -Brief	cross-sectional study	• Psychological domain scored highest, followed by social, environmental, and physical. • Quality of life satisfaction linked to psychosocial factors and emotional support. • No significant gender difference in quality of life domains among amputees. • Physical domain had the lowest score, while psychological domain scored highest.	Malaysian lower limb amputees' quality of life is satisfactory, with physical health scoring low but still satisfactory, supported by cultural and psychosocial support and rehabilitation facilities.

Table Continued

Magnusson et al., (December 2019) India/ Biomedical Centre [25].	N- 30 (100%) Age (Mean) –37.5 Gender Male 15(51%) Female- 15 (49%) Marital Status-N/S Education Not attended school 4 (12%) Attended school -26 (88%) Amputation cause- Accident 16 (53.33%) Gangrene 10 (33.33%) Other 4 (13.33%) Amputation types- TT -19 (63.33 %) TF - 11 (36.66 % Prosthesis user's -30 (100%).	Inclusion- Participants with lower-limb physical disabilities who received prosthetic or orthotic services from Mobility India within the last three years were included in the disability group. Exclusion A serious mental illness, severe cognitive impairment, blindness, or deafness were the exclusion criteria. The study did not include any staff or students from Mobility India who used orthotic and prosthetic devices.	WHOQOL -Brief	cross-sectional study	• Physical disability linked to lower QOL in multiple domains. • Income, education, and living area influence QOL scores. • Urban slum residents face higher risk of low QOL • Gender, income, and education impact QOL in various domains.	The physical, psychological, and environmental domains of quality of life (QOL) are all adversely affected by physical disabilities. Longer education has a beneficial effect on QOL, however income has an impact on QOL in terms of psychology and surroundings.
Alessa et al., (November 2022), Saudi Arbia/ Cureus Inc. [27]	N- 239 Age(Mean)- 37 year Gender Male 167 (69.9%) Female 72 (30.1%) Marital status Married 113 (47.3%) Single 91 (38.1%) Divorced / widow 35 (14.6%) Education- Below secondary/ secondary 157 (65.7%) University / above 82(34.3%) Level of amputation- TT-101 (42.3%) TF/KD- 104 (43.6%) Bil.-34 (14.2%) Cause of amputation Trauma- 94 (39.3%) Infection /diabetes/	The study excluded participants under under the age of 18.	TAPES, Depression, Anxiety, Stress Scale (DASS) Caregiver-Related Question. Zarit Burden Interview (12-item ZBI) and Short-Form Health Survey (SF-12)	cross-sectional study.	• Prosthetics users had higher QOL scores than non -users. • Caregivers experienced varying levels of burden, with 15.1% high burden. • Psychological adjustment and prosthesis satisfaction correlated with QOL dimensions.	The study emphasizes the value of psychological assessment and counselling in amputee treatment by finding a link between TAPES and SF-12 PCS and MCS scores.

Table Continued

	vascular- 96 (40.2 %) Cancer- 17 (7.1%) Others- 32 (13.4%) Prosthesis user's Yes- 104 (43.5%)					
Deepak et al., (March 2023), India/ Cureus [28]	N- 106 Age (Mean)- 40 year Gender- Male-83(78.30%) Female- 23 (21.70%) Marital status- N/S Education- Illiterate- 24.99 (23.58%) school- 49.98 (47.16 %) beyond high school- 29.25%, Amputation level- TT- 67 (63.31%) TF- 37 (34.91%) Other- 2 (1.89%) Amputation cause- RTA- 73 (68.87%) DM/PVD-17 (16.04 %) Tumor-9 (8.49%) Other cause-7 (6.60%) Prosthesis user's- 65 (37.75%).	Inclusion- The study aims to involve 18+ patients with unilateral lower limb amputation, post-amputation patients over three months, willing to participate and provide informed consent. Exclusion-Patients with amputation, post-amputations under three months, refusal to participate, hearing, speech, visual function disorders, polytrauma, and psychiatric illness are excluded.	WHOQOL -Brief	cross-sectional study.	• Physical domain most affected, followed by psychological, social, environmental domains. • Trauma top cause of amputation, followed by diabetes, cancer, vascular disease. • Transtibial amputees more common than transfemoral. • Majority of patients aged 20-39 years.	Delays in prosthesis fitment exacerbate amputees' physical burden, while early prosthesis and psychological counselling can significantly improve their quality of life.
Priyadharshan et al. (Jan-2022), India/ Prosthetics & Orthotics international [29].	N- 106 Age (Mean) - 51.42 year Gender- Male-88(83%) Female- 18 (17%) Marital status -N/S Education- N/S Amputation level TT – 71 (67%) TF/KD/FF – 35 (33 %) Amputation cause- TA- 44 (41.5%) DM/PVD- 61 (57.6 %) Other cause-1	Inclusion: People who have lost a lower limb and are at least eighteen years of age and have had surgery within the last five years are eligible to participate in the survey. Exclusion: Amputees who suffer from any type of mental illness or chronic diseases such as	WHOQOL -Brief, TAPES -R	cross-sectional study.	• Amputation has a substantial impact on every aspect of life quality. • QOL is impacted by age, time since amputation, and prosthetic use. • Individuals who use prosthetic devices live	Amputation significantly impacts quality of life (QOL), with age affecting QOL. Age-related QOL decreases, but 48.1% use prosthetic devices, with better QOL. Residual and phantom limb pain prevalent.

Table Continued

	(0.9%) Prosthesis user's- Yes- 51(48.1%)	cancer, AIDS, or kidney problems may have to have several little or major toe amputations.			far better lives.	

Abbreviations: N: number of participants; TT: transtibial; TF: transfemoral; Bil: bilateral; KD: knee disarticulation; FF: forefoot; RTA: road traffic accident. DM: diabetes mellitus; Peripheral vascular disease (PVD) QoL: quality of life; RAND-36: Research and Development Corporation Measure of Quality of Life; HRQOL: health-related quality of life; SF-12: 12-item Short Form Survey; WHOQOL-BREF: World Health Organization Quality of Life-BREF; MOS: Medical Outcome Study; TAPES: Trinity Amputation and Prosthetic Experience Scale; PEQ: Prosthetic Evaluation Questionnaire; N/S: not stated; ©: Number of citations.

CONCLUSION

Lower limb amputations profoundly impact individuals' physical, psychological, and social well-being. This review highlights the critical factors influencing QoL, including demographic characteristics, cause and level of amputation, prosthetic use, and psychosocial support. Effective rehabilitation must address these multifaceted needs, ensuring that amputees receive comprehensive care to enhance their overall quality of life. Future research should focus on long-term outcomes and include a broader range of geographical and cultural contexts to develop globally applicable rehabilitation strategies.By understanding the diverse factors influencing QoL among lower limb amputees, healthcare providers and policymakers can develop more effective, targeted interventions that address the unique needs of this population, ultimately improving their rehabilitation outcomes and overall quality of life.

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Date : 31st June 2024

Place : Bhopal

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