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Editors Communique

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
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Evidence of Pain Neuroscience Education on Musculoskeletal Disorders: An Updated Review

Sunidhi,¹ Sidhant,² Bhawna Verma,³ Megha Gakhar,⁴ Ramta Devi⁵ and Poonam Devi⁶

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ABSTRACT

Pain neuroscience education (PNE) is a new concept which helps the patients to modulate their pain perception and experiences. The concept has shown effectiveness, comparable to other pain-relieving modalities including manual therapies. The objective of the current review is to explore the mechanism on which pain neurosciences work and various methodologies to implement pain neuroscience education. The current literature of pain neurosciences education has been reviewed and the evidences synthesised to explore the above objectives. Pain neuroscience education takes into account the complexity of a pain experience and aids patients in redefining pain by educating them on the various neurophysiological, neurobiological, social, and physical factors that may be present in their particular pain experience. The main objective of PNE is to assist individuals in rethinking their chronic pain. The mechanism through which PNE works is reframing the way we think about pain. It is important to accept that in chronic pain, pain is not a direct measure of tissue damage and more chronic the pain it becomes less reliable gauge for tissue damage. It is also important to note that pain is influenced by physical, psychological and social factors. According to research, pain neuroscience education is a successful therapy technique for a number of chronic pain subtypes, including whiplash-related pain, neck pain in teenagers, and pain from lumbar surgery, complicated regional pain syndrome, and low back pain. PNE has advantages for participants from early adolescence to old persons. Pain Neuroscience Education is an effective method of managing pain and may be used by the physiotherapist to manage the patients with musculoskeletal conditions. Chronic musculoskeletal pain is very common complaint and research shows that pain neuroscience education is effective in pain management and physiotherapists should utilize the concept in patients as it improves patient engagement in health care.

KEY WORDS: CATASTROPHIZATION, CHRONIC MUSCULOSKELETAL PAIN, PAIN BIOLOGY EDUCATION, PAIN NEUROSCIENCE EDUCATION, RECONCEPTUALIZATION.

INTRODUCTION

The first mention of pain neuroscience education (PNE) in literature was in 2002 in patients with low back pain. (Meeus 2010). The terms pain biology education (PBE) as well as pain neurophysiology education have been used interchangeably with pain neuroscience education in literature, (Meeus 2010 and King 2016). Chronic pain is different from acute pain in its physiology and biopsychosocial impacts. Empowering the patients with knowledge regarding chronic pain mechanisms and neurophysiology alters patient's fundamental perception regarding chronic pain, (Butler 2003, Eneberg-Boldon 2020 Alfaifi and Webb 2021).

Goudman (2019) state that pain is a commonly used term for PNE and the textbook with same title serves as foundation

for PNE. The goal of PNE is to educate patients on the principles of neurophysiology of pain, nervous system sensitization, spinal inhibitory mechanisms, nervous system plasticity, and psychosocial factors that affect the experience of persistent pain, (Louw 2016, Meeus 2010, Mosely 2002 & King 2016).

The key underlying mechanism through which PNE is thought to function is re-conceptualization, which is described as the development of a new, less dangerous understanding of the nature of one's pain. In cases of chronic pain, it is important to realise that pain is not proportional to tissue injury. Chronic pain is greatly influenced by psychological and social factors, (Moseley 2007). PNE as a technique is administered by qualified healthcare professionals. It can be carried out separately or in groups. The neurophysiological concepts of chronic pain are explained to patients in simple language with the use of appealing and interesting freehand illustrations and metaphors.

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As a treatment PNE can be used alone but most frequently it serve as a foundation for comprehensive pain treatment strategy. Emerging research supports that PNE may be helpful for pain and function in the physical, psychological, and social realms, (Ryan 2010, (Moseley 2007, Moseley 2015, Moseley 2004, Louw 2011). The concept of PNE has gained importance in chronic musculoskeletal pain management. Recent years have seen an urge in literature regarding PNE. The present review was aimed to explore the methodologies through which PNE is implemented and the evidences for its effectiveness.

MATERIAL AND METHODS

An electronic search was performed covering the last 2 decades (2003 –2022). The main search items were neuroscience, neurobiology, neurophysiology, pain, pain education, pain science, education, musculoskeletal

disorders. All of the identified literature was reviewed for titles and abstracts. The selected studies on musculoskeletal pain were reviewed.

RESULTS AND DISCUSSION

Strong evidences in support of pain neuroscience education are coming up. Patients with pain are often interested in knowing what causes their pain. The biomedical education commonly uses ergonomics including anatomy, biomechanics and pathoanatomy overlooks the complex issues like central sensitization, peripheral sensitization, inhibition and neuroplasticity. Sometimes these are taken up by patients negatively and develop into fear avoidance and catastrophization. PNE embraces a biopsychosocial approach. One of the important aspects to note in PNE is pain experience. The review suggests that patients treated with PNE have less fear avoidance and catastrophizing behaviour.

Table 1. Research studies on PNE in musculoskeletal disorders

Sr. No	Author, year	Study design	Patient Population	PNE Methodology	Results
1	Meeus (2010)	RCT	Chronic fatigue syndrome	30-minute lecture on pain physiology	Pain physiology education can be a significant therapeutic strategy in the treatment of individuals with chronic fatigue syndrome and chronic pain.
2	Louw (2011)	Systemic review	Chronic musculoskeletal pain	Studies on PNE published in English language from 1999-2010	Provide strong support for the idea that both passive and active physical movements are impacted by NE.
3	Kinga (2016)	Qualitative study	Chronic musculoskeletal pain	3 Weeks pain neuroscience education	Support the idea of reconceptualization is a key process and offer practical guidance for the work required to further explore this.
4	Shepherd (2018)	A Case Report	Complex Regional Pain Syndrome (CRPS)	A total of 26 sessions on pain neuroscience education (PNE), laterality training, motor imagery, and mirror training were conducted over a nine-month period.	Long-term outcomes showed little to no fear-avoidance and catastrophizing behaviours, as well as no functional restrictions related to her foot or ankle.
5	Goudman (2019)	A Clinical perspective	Patients Undergoing Surgery for Lumbar Radiculopathy	Before and after the surgery, 2 personally customised PNE sessions (each lasting about an hour), in addition to an educational brochure.	Physical therapists can use the clinical perspective to help them implement perioperative PNE to patients having surgery for lumbar radiculopathy.
6	Watson (2019)	A mixed-methods systematic review and meta-analysis	Chronic musculoskeletal pain	Four qualitative studies (n = 50) examining patients' experiences with PNE	Data from 12 RCTs showed that PNE can, in the short-to medium-term, improve

				were included together with 12 randomised controlled trials (n = 755) reporting pain, disability, and psychosocial effects.	pain, disability, pain catastrophizing, and kinesiophobia. Data from four qualitative investigations revealed a number of crucial factors that can improve patients' experiences with PNE, such as letting patients share their own stories.
7	Corissa (2021)	Pre and post survey	Chronic pain	9 out of the 26 participants who successfully completed both the before- and after-PNE questionnaires were included in the quantitative survey results. 5 responses only were gathered for the qualitative data.	In comparison to pre-PNE scores, post-PNE scores demonstrated a statistically significant ($p = 0.02$) improvement. The qualitative aspect of this investigation showed that participants did indeed redefine pain.
8	Oosterwijk (2011)	A pilot study	Chronic whiplash associated disorders	A 30 minutes 2 education session on PNE in 2 groups is given	Data from chronic whiplash-related illnesses indicate that learning about pain physiology is associated with improvements in pain cognitions, pain thresholds, and pain-free movement performance.
9	Moseley (2003)	A randomized comparative trial	Chronic low back pain	Group education (GE) featured a single 4-hour education session with a group of 7–10 patients, while individual education (IE) involved four 1-hour teaching sessions.	The findings showed that an intervention based on a cognition-specific motor control training method combined with pain physiology education is efficient in reducing pain and impairment related to chronic LBP.
10	Alfaifi and Webb (2021)	A Case Study	Chronic plantar fasciitis pain	PNE was applied for 5 to 15 minutes of treatment with 4 other interventions. 1st was Extracorporeal Shock Wave Therapy and 2nd was the mobilization grade III and VI were used for 5 minutes every session and 3rd was Low dye taping and 4th was stretching exercises for the lower limbs.	The symptoms in this case were not entirely resolved, the patient did report improvements in their symptoms and functional abilities, which gives hope that this case may be a useful addition to future therapies.
11	Nito (2018)	Qualitative study followed a quantitative, randomized, and controlled trial	Chronic idiopathic neck pain	43 students in one high school's 10th to 12th grades were involved; 21 were in the experimental group and received pain neuroscience instruction along with neck and shoulder endurance exercises, whereas 22 were in the control group and did not get any intervention.	Adolescents with chronic idiopathic neck pain approve the intervention, which consists of education about pain neurology and exercise, and they find it relevant and suitable.

PNE, by its definition, is an educational intervention aiming to alter a patient's beliefs and cognitions regarding their pain experience, (Louw 2017). The education of pain neurophysiology goes hand in hand with de-education the common myths about the condition. PNE is usually delivered through lectures and interactive sessions. It includes pictures, stories, examples and metaphors to promote a positive and deep learning. In order to implement the PNE it becomes essential to identify various biopsychosocial factors in patients with chronic pain. Hence, somatic, cognitive, emotional, behavioural, social and motivational factors must be included in pain assessment, (Amarins 2016). This may help to identify the dominant pain mechanism (nociceptive, neuropathic and central sensitization pain).

The mechanism through which PNE works is reframing the way we think about pain. It is important to accept that in chronic pain, pain is not a direct measure of tissue damage and more chronic the pain it becomes less reliable gauge for tissue damage. It is also important to note that pain is influenced by physical, psychological and social factors. The coping and belief of patient improves due to reconceptualization of pain. This restores balance in pain mechanism and pain inhibition improves which ultimately improves health status and functioning.

The review shows level A evidence for the effectiveness of PNE in chronic musculoskeletal pain as supported by RCTs and meta-analysis. However, clinicians must consider proper assessment of pain experiences. The patients who have poor current concepts will improve with reconceptualization. Imparting PNE in language and method understandable to patient is the key to its effectiveness.

CONCLUSION

Pain Neuroscience Education is an effective method of managing pain and may be used by the physiotherapist to manage the patients with musculoskeletal conditions. Chronic musculoskeletal pain is a relatively frequent complaint, and studies have shown that education in pain neurology is useful in treating it. Physiotherapists should use this idea with patients since it increases patient involvement in their care.

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Modeling of a Positive Case of Covid-19 Through Regressive Objective Regression Without Doing PCR

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ABSTRACT

Currently, new technological advances in biomedicine make the creation of multidisciplinary teams of vital importance. These groups can be made up of clinicians, epidemiologists, mathematicians, statisticians, computer scientists, biologists, among others, all together they can achieve an accurate prediction of infectious diseases and thus draw up the appropriate strategies by the competent authorities. The fundamental objective of this work is to obtain, through Regressive Objective Regression (ROR), the modeling of the next positive case that arrived with COVID-19 without performing PCR at the "Marta Abreu" Trashing Polyclinic in the city of Santa Clara. In this work, daily data were used from January to March corresponding to the year 2021 of the number of Covid-19 cases in the "Marta Abreu" Teaching Polyclinic in the city of Santa Clara, in the province of Villa Clara, Cuba, a total of 3294 cases of them 58 positive, of which they are assigned in the database an order number (No) according to how they were registered in the database. In the short-term modeling, the model was assigned to 19.7% with an error of 0.12 the dichotomous variables, saw tooth and inverted saw tooth, and the risk returned in 1.3 and 12 cases, the trend is negative and not significant. The ROR modeling of predictions obtained give very significant results for the study of the COVID-19 pandemic at the Marta Abreu Teaching Polyclinic. With the results of the study, the authorities are provided, and in fact they are already doing so, with information on the short- and medium-term behavior of variables of great interest to understand the expansion of SARS-CoV2, which could be used for decision-marking.

KEY WORDS: CORONAVIRUS; COVID-19; MATHEMATICAL MODELING; ROR MODEL.

INTRODUCTION

Infectious diseases have historically had a great impact on morbidity and mortality (Gubler, 2002), which surely led to the prediction of the evolution of epidemics for centuries (Osés et al., 2017).

The great development acquired by different sciences, such as epidemiology, mathematics, statistics, computer science, has led to the current generation of new techniques in emerging and recurrent procedures in the field of biostatistics

and bioinformatics (spatial statistics, neural networks and functional data analysis, among others) to describe the importance of mathematical models in infectious diseases (Casals & Caylà, 2009). During this anti-epidemic battle, next to medical and biological research, theoretical studies based on statistical or mathematical models can also play a non-negligible role in understanding the characteristics of the epidemic, in predicting the tipping point and the final time future potential growth, help to estimate the risk to other countries and in deciding on measures that contribute to curb the spread of the disease (Funk et al., 2017). Estimating changes in transmission over time can provide insight into the epidemiological situation, and the

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estimate identifies whether control measures are having a desired effect (Vilches, 2020).

The use of predictive statistical models in the health sciences has grown significantly in recent years (Cortellis, 2020; Ivorra & Ramos, 2020; Osés et al., 2020). These emerge as an important link between statistics and medical practice; they are of great help in decision-making and allow the creation of various systems and tools useful to reduce uncertainties, ensure better performances and establish effective control measures for diseases (López et al., 2016; Osés et al., 2020).

Analyses of predictions can inform about future potential growth and helps to estimate risk to other countries (Funk et al., 2017). Statistical predictive models have been using for predictions of the likely evolution of COVID-19, among which can be found moving average model, automatic regression integrated (ARIMA), Brown / Holt linear exponential smoothing methods and Objective Regression-ROR methodology (Osés et al., 2020; Prades & Martin, 2020; Vilches, 2020). Scientists around the world began to work on the mathematical modeling of the epidemic caused by the new SARS-CoV-2 coronavirus causing COVID-19, using various techniques and mathematical models (Osés et al., 2020; Sun et al., 2020; Yi et al., 2020; Osés et al., 2021a,b). Different avenues have been used for such mathematical modeling: classical modeling for epidemics with SIR models based on ordinary differential equations (Bacaër, 2020; Cagigal & Becario, 2020; Dayun et al., 2020).

In the modeling of COVID-19, statistical time series models, predictive models using the Internet, and models based on artificial intelligence and Machine Learning stand out. The aforementioned models take into account a series of parameters that allow the inclusion of several factors in order to express the realities of the epidemics as well as possible (Dayun et al., 2020; Medina, 2020; Osés et al., 2020). The main objective of the present work was to obtain, by means of the Objective Regression Regressive (ORR), the modeling of the next positive case that arrives with Covid-19 without PCR at the "Marta Abreu" polyclinic in the city of Santa Clara, Villa Clara, Cuba.

MATERIAL AND METHODS

In this study, the daily data from January to March 2021 were used for the number of COVID-19 cases from the "Marta Abreu" Polyclinic, located in the city of Santa Clara, Villa Clara province, Cuba. Of 3 294 cases, 58 of them were positive, of which an order number (No) was assigned in the database, as they were registered in the database. These data were taken from the statistics department of the polyclinic referred to above. The forecast was performed with the use of the methodology of Regressive Objective Regression (ROR), which has been implemented in different variables, such as viruses and bacteria circulating in Villa Clara province (Osés, 2004; Osés & Grau, 2011).

The Regressive Objective modeling (ROR) is based on a combination of Dummy variables with ARIMA modeling,

where only two Dummy variables are created and the trend of the series is obtained; it requires few cases to be used and also allows using exogenous variables that make it possible to model and forecast in the long term, depending on the exogenous variable; it has given better results than ARIMA in some variables, such as HIV modeling, entities of viral etiology/arbovirosis and parasitic entities (Osés & Grau, 2011; Fimia et al. , 2017; Osés et al., 2020).

In the ROR methodology, in a first step, dichotomous variables DS, DI and NoC are created, where: NoC: Number of cases of the base, DS = 1, if NoC is odd; DI = 0, if NoC is even, when DI=1, DS=0 and vice versa. Afterwards, the regression analysis is performed with the SPSS version 19.0 statistical package (IBM Company), specifically the ENTER method where the predicted variable and the ERROR are obtained. Then the autocorrelograms of the variable ERROR will be obtained, paying attention to the maximums of the significant partial autocorrelations PACF. The new variables are then calculated according to the significant Lag of the PACF. Finally, these regressed variables are included in the new regressions in a process of successive approximations until white noise in the regression errors is obtained.

The research was subject to ethical standards, where all the information collected and provided was used only for the stated purpose. It did not involve physical or psychological affectations, in order to be able to generate new knowledge without violating the ethical principles established for these cases. On the other hand, all authors involved in the research, publication and dissemination of the results are responsible for the reliability and accuracy of the results shown (DHAMM, 2013).

RESULTS AND DISCUSSION

The data on COVID-19 confirmed cases at the "Marta Abreu" teaching polyclinic are shown in Table 1. The risk of contracting COVID-19 was calculated by descriptive statistics, where a value of zero was assigned if the PCR was negative, and if the PCR was positive, a value of Risk 1 was assigned (Table 1), showing a mean value of 0.0176 with a standard deviation of 0.13.

Table 1. COVID-19 confirmed at the "Marta Abreu" teaching polyclinic during the year 2021

	Descriptive statistics				
	N	Minim.	Max.	Half	Typ. desvia.
Risk Covid-19	3294	.00	1.00	.0176	.13154
N valid (according to list)	3294				

Another result obtained refers to the distribution according to sex, 54% of the patients corresponded to the female sex (Figure 1). The mean value of this variable corresponds to 19.33 cases with a standard deviation of 12.01 cases.

Figure 1: Distribution of patients according to sex in the "Marta Abreu" polyclinic. January to March 2021

Distribution of patients according to sex.
Marta Abreu Polyclinic. Santa Clara
January-March 2021



Table 2. Summary of the model using ROR Regression

Summary of model ^{c,d}					
Model	R	R squared ^b	R squared corrected	Standard error of estimation	Durbin-Watson
1	.999 ^a	.998	.998	80.4879	2.132

a. Predictors: Lag5No, DS, DI, Lag12No, Lag1No, NoC

b. For regression through the origin (the model without intercept), R-squared measures the proportion of the variability in the dependent variable about the origin explained by the regression. This CANNOT be compared to R-squared for models that include intercept.

c. Dependent variable: No

d. Linear regression through the origin.

The COVID-19 cases were modeled in the short term using the ROR methodology. Table 2 shows how 99.9% of the variance is explained with an error of 80 cases; the Durbin Watson statistic is close to 2, so we are in the presence of a model that adequately explains the variance.

The analysis of variance of the ANOVA model for COVID-19 is shown in table 3. Fisher's test had a value of 4066.33, interpreted as significant at 100%, thus proving that the sample variances are not equal.

The ROR model in question (Table 4) is made up of the following variables, DI and DS which are dichotomous variables, saw tooth DS and inverted saw tooth DI and of the Order number of the patient returned in 1.5, and 12 cases (Lag1No; Lag5No and Lag12No, the trend is positive because the number of patient is increasing and is significant at 99%.

The concordance between the Actual value of the Patient Order Number (No) and the non-standardized predicted value can be appreciated (Figure 2). The actual and predicted value of the positive patient number allows for more accurate surveillance of COVID-19 positive cases without PCR. As can be seen there are highs and lows that the model describes with certainty.

DISCUSSION

The modeling of epidemics is something historical and has been improved with the advance of science and technologies, which demonstrates the essential role of mathematics to indicate where the disease can move and give suggestions on how to decide (Vidal et al., 2020).

Table 3. Analysis of Variance of the model for COVID-19

ANOVA ^{a,b}					
Model	Sum of squared	gl	Quadratic mean	F	Sig.
1 Regression	158057429.217	6	26342904.870	4066.333	.000 ^c
Residuo	259131.783	40	6478.295		
Total	158316561.000 ^d	46			

a. Dependent variable: No

b. Linear regression through the origin

c. Predictors: Lag5No, SD, DI, Lag12No, Lag1No, NoC

d. This total sum of squares is not corrected for the constant because the constant is zero for regression through the origin.

COVID-2019 disease has been recognized as a global threat, in which predictive models for the epidemiological trend of its prevalence and incidence have been used worldwide, some of the models used being: Brown's exponential smoothing used in Russia, and time series models (ARIMA) used in Turkey, Germany, France, Italy, Thailand, China, South Korea and Iran (Prades & Martin, 2020).

When analyzing the predictions for the confirmed cases to COVID-19 in the Teaching Polyclinic "Marta Abreu" without doing PCR through the Objective Regressive Regression, we can appreciate that the mathematical model has followed what happened in reality, this is the most important result, which coincides with results obtained in previous years for other entities and living organisms

(Fimia et al., 2017; Osés et al., 2017; Fimia et al., 2019), agreeing also, with other models applied for COVID-19 in other countries (Prem et al., 2020). By analyzing the concordance between the Actual value of the Patient Order Number (No) and the non-standardized predicted value, it

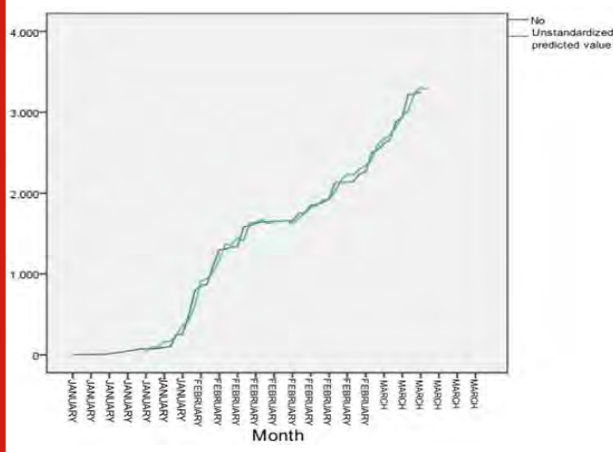
allowed to determine the actual and predicted value of the number of the positive patient without doing PCR. These results are in agreement with similar studies carried out in Villa Clara province (Osés et al., 2020).

Table 4. Conformation of the ROR model with its variables

Model	Coefficients ^{a,b}					
		Unstandardized		Standardized	t	Sig.
		B	Standard error	Beta		
1	DS	-438.903	135.185	-.167	-3.247	.002
	DI	-400.395	137.034	-.153	-2.922	.006
	Tendency	31.932	8.825	.652	3.618	.001
	Lag12No	-.206	.082	-.136	-2.507	.016
	Lag1No	.799	.105	.772	7.641	.000
	Lag5No	-.108	.082	-.090	-1.308	.198

a. Dependent variable: No
 b. Linear regression through the origin

Figure 2: Concordance between the Actual value of the Patient Order Number (No) and the unstandardized predicted value



The increase in the number of female patients infected by the new coronavirus in the mentioned health area shows that they are as susceptible to the disease as the rest of the population. They should all follow the same biosecurity measures as the Ministry of Public Health for the entire population. It coincides with the daily reports of MINSAP, that the female sex is the one with the highest number of cases reported in recent weeks (MINSAP, 2021).

All that has been analyzed shows us that teamwork is essential to achieve success in the confrontation and control of this pandemic that today plagues the planet and even the exchange of experiences and results between professionals of different specialties (Abdullah et al., 2020), working

groups of different specialties and branches of scientific knowledge and geographical latitudes, with emphasis between groups in our area and the region is essential in the current epidemiological context, due to climatic and sociocultural similarities in many cases (Fimia et al., 2019; Marín et al., 2020; Salas et al., 2020).

CONCLUSION

The ROR modeling in function of the next positive case with arrival in Santa Clara without PCR, gave very significant results for the study of the COVID-19 pandemic in the "Marta Abreu" polyclinic, so that the results of the study provide the authorities with information on the short and medium term behavior of variables of great interest to understand the expansion of SARS-CoV2, which would be a good tool for making timely and correct decisions.

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First Survey and Identification of Forensically Important Entomofauna in Makkah City

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ABSTRACT

Most of the murders are shrouded in mystery and the absence of a lot of forensic evidence that helps the authorities achieve justice and reveal the culprit. The insects present at the crime scene and their various stages remain clues and evidence that are very important in knowing and revealing the circumstances of the incident. Therefore, determining the species of insect related to forensic medicine is very important for each region separately. The present study was planned to survey of forensic insects species and its seasonal activity in Makkah governorate. The local white rabbits were used as a mammal closer to the human corpse. The work was carried out of one year from January 2007 to December 2019. During the survey, 15,757 flies of importance forensic insects were collected which represent three families: Muscidae (47.1%)•Calliphoridae (30.0%); Sarcophagidae (22.6%), and Muscidae (47.1%). In this study, four flies species were reported for the first time in Makkah City in the Kingdom of Saudi Arabia, these were *Chrysomya albiceps*; *Ch. marginalis*; *Sarcophaga dux* and *Wohlfahrtia bella*. The appearance of the *W. bella* species was first recorded in Saudi Arabia. The results showed the presence of forensic insects most of the time during the year, and there were significant differences in different months of the year, and the reason for this may be due to differences in climatic conditions, temperature, and relative humidity which are considered among the most important factors affecting the reproduction and spread of the insects.

KEY WORDS: CALLIPHORIDAE; FORENSIC INSECTS; MAKKAH; SARCOPHAGIDAE; SURVEY.

INTRODUCTION

The science of forensic entomology is a comprehensive field in which arthropods and the judicial system interact. This science involves the significant application of insects and other arthropods to aid in legal investigations. Some species of insects have potential value in solving crimes, in terms of determining post-mortem interval (PMI), being

the minimum time between death and the discovery of a body, and achieving justice through their relevance to legal medicine, for example in cases of a suspicious death, and/or by analysing the composition of insect species on a cadaver (Carvalho et al., 2004; Niederegger et al., 2012). This science plays an important role in determining the circumstances of crime and causes of death (Catts and Goff, 1992; Sukontason et al., 2007; Khairou, 2022).

There are many species of blow flies (Diptera: Calliphoridae), including *Lucilia sericata*, *Chrysomya albiceps*, and *Calliphora vicina*, which can be used to detect some of the

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circumstances around a crime as these flies are often the first to arrive on a dead body, where they can lay their eggs and hatch into larvae that feed and are reared on the corpse (Smith, 1986; Higley and Haskell, 2009). There are other important species of flesh fly (Diptera: Sarcophagidae), such as *Sarcophaga* sp., which provide significant information about the circumstances of crime (Grassberger and Reiter, 2002; Amoudi et al., 1994; Khairou, 2022).

There is a scarcity of environmental studies related to forensic entomology in the Kingdom of Saudi Arabia, which highlights the importance of the present study in enumerating the types of forensically important insects, defining them at the level of gender and species, and linking this information with the stages of decomposition of the body, as well as studying the seasonal correlations (autumn, winter, spring, and summer) and fluctuations in their population dynamics, and determining the quality of the correlation between population density and the prevailing climatic conditions in the city of Makkah over a year.

of Meteorology and Environmental protection in Saudi Arabia. This data covered the whole of 2019, from January to December. The survey area was divided into several locations including the north, south, east, and center of Makkah (Fig 1).

Figure 1: The site of collecting samples

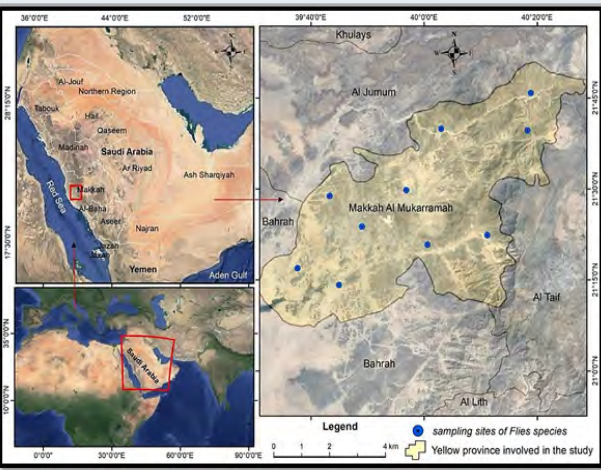


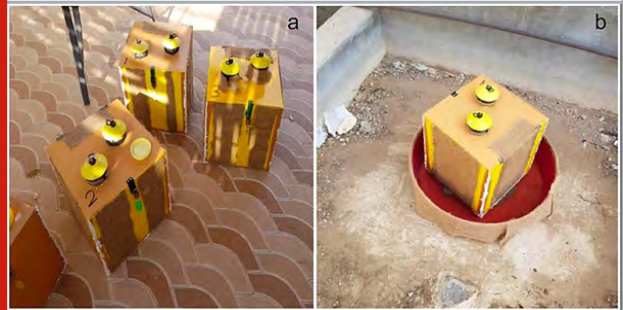
Figure 2: The final flight trap to implement this experiment with high efficiency: the bottom of the traps was removed, and a cloth basket was inserted to collect samples.



MATERIAL AND METHODS

This study aimed to identify the diversity and abundance of forensically important insects in the city of Makkah, which is located at the latitude and longitude coordinates 21°25' N and 39°49' W, respectively. The weather data used in this research was obtained from the General Authority

Figure 3. a: Each cage with two holes on the upper side in which to place the modified final flight traps; b: Closed with a cement block from the lower side after placing the corpses inside the acrylic cages, then putting in plastic basins to prevent ants from reaching the cadavers.



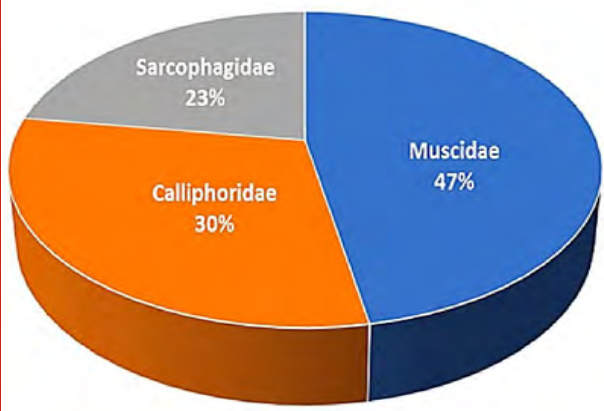
Cages and modifications: A ‘final flight’ trap was used in this experiment, which is a type of baited trap. The traps contain rabbit corpses of *Oryctolagus cuniculus* that were chosen to mimic the normal death case (human dead body). The rabbits were obtained from local shops in Makkah city, then killed with chloroform for this study. Some modifications were made to the final flight trap to implement this experiment with high efficiency: the bottom of the traps was removed and a cloth basket was inserted to collect samples (Fig. 2). Two of the traps were placed inside a cage made of acrylic (50 × 50 × 50 cm). The cage had two holes on the upper side in which to place the modified final flight traps (Fig. 3A). The acrylic cages were closed with a cement block from the lower side after placing the corpses inside. Then, the cages were put in plastic basins to prevent ants from reaching the cadavers (Fig. 3B).

Insect collection and classification: Insects were collected each day by carefully replacing the cloth basket, taking out the old one full of insects, and replacing it with a new one. The removed cloth baskets were full of live insects. Therefore, they were killed by freezing (-6°C) for one hour. Then, the specimens were incubated at room temperature for 10 minutes to stretch their muscles to preserve them (pinning). The samples were initially identified based on their morphological characteristics, depending on taxonomic description keys (Borror et al., 1981) under a stereo light microscope (Leica EZ4 D) with a digital camera (B41 Olympus fitted with magnification power 32X and variable magnification) at the Insect Classification Unit in King Abdul-Aziz University. To aid specimen classification, some of the samples were put into labelled vials with Ethanol mixed with water (70% to 80%) and were sent to King Saud University to be classified by Professor Hathal, who used valid taxonomic keys (Borror et al., 1981).

Statistical analysis: A comparison of the means of the population densities of forensically important flies was

carried out using General Linear Models Procedure. All analyses were undertaken using SAS 2001 for Windows (IBM, Version 24) and all figures were drawn using Excel (Microsoft Corp, USA).

Figure 4: The highest number of Muscidae with 7,417 flies forming 47.1 % and followed by Calliphoridae, with 4,773 samples forming 30.0% of the total, and finally the Sarcophagidae family, with 3,567 flies total and 22.6% of the total.

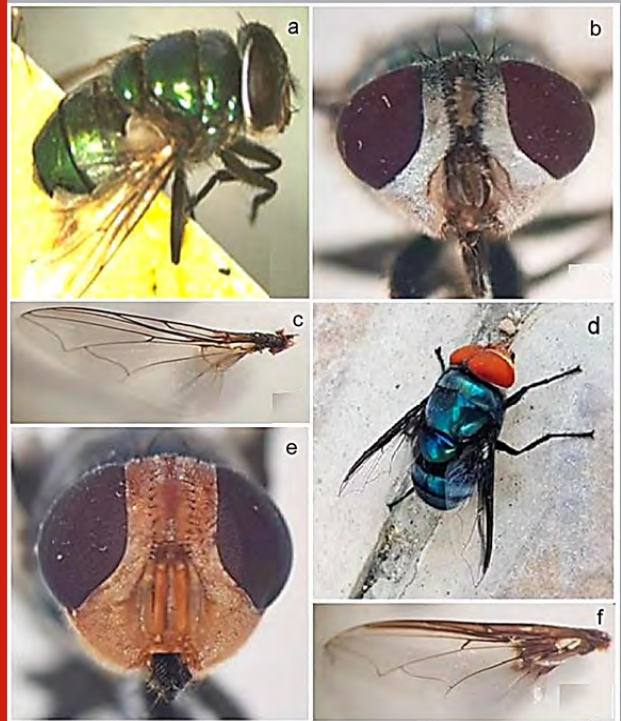


RESULTS AND DISCUSSION

The study aimed to investigate the diversity and abundance of forensically important insects during 2019 (from January to December) in the city of Makkah, west Saudi Arabia. The results identified five different species of Diptera, which were recorded for the first time in Makkah. The total number of different species of adult Diptera collected was 15,757 samples, across the three families of Sarcophagidae, Calliphoridae, and Muscidae. The number of Muscidae collected was 7,417 flies, representing the highest percentage of the total, at 47.1%, followed by Calliphoridae, with 4,773 samples forming 30.0% of the total, and finally the Sarcophagidae family, with 3,567 flies total and 22.6% of the total (Fig. 4).

Calliphoridae family: In this part of the study, two species of Calliphoridae were recorded, belonging to the *Chrysomya* genus. Within species, *Chrysomya albiceps* (Wiedemann, 1817) was the most abundant and prevalent, at 87%, compared to *Chrysomya marginalis* (Wiedemann, 1830), at 13%. In regard to species description, *Ch. albiceps* is characterised by a metallic green colour, with silver frons and a single pair of membranous wings without any pigmentation. Whereas *Ch. marginalis* is characterised by metallic blue, and orange colour frons, and possesses a darkened anterior wing margin costal (C) and subcostal (Sc), the main defining feature used to identify this species (Fig. 5). Although, these species were recorded for the first time in Makkah, they have been identified in studies in many other cities in Saudi Arabia (Al-Shareef and Al-Qurashi, 2016; Al-Qurashi, 2016; Al-Shareef et al., 2016; Ali Khan et al., 2018; Al-Jameeli et al., 2021; Khairou, 2022).

Figure 5: Classification of morphological characteristics, depending on keys of taxonomic descriptions under a digital stereo microscope; A: *Ch. albiceps* is characterised by a metallic green colour, B: with silver frons, and C: a single pair of membranous wings without any pigmentation; D: *Ch. marginalis* is characterised by metallic blue, E: orange colour frons, and F: possesses a darkened anterior wing margin costal (C) and subcostal (Sc).



Regarding the Calliphoridae population, the findings showed that three peaks were recorded for this family in a year, over different seasons. The highest population by a significant margin was found in June, whereas the lowest was in November (Fig. 6). The third peak was in March, which was higher than November, but not significantly (Fig. 6). There were significant differences in Calliphoridae abundance across different seasons (Table 1). The other purpose of this part of the study was to investigate the effect of abiotic factors (temperature and humidity) on the population of Calliphoridae. The results showed a negative correlation, but not a significant one, between temperature and Calliphoridae population ($R = -0.136$, $P = 0.52$). The relationship between humidity and the population of Calliphoridae was positive but not significant ($R = +0.181$, $P = 0.397$).

In regard to attraction, the results showed that the number of Calliphoridae forensic flies was significantly higher and they were more attracted to the corpse immediately after death; in the early decomposition stage (fresh); followed by the bloating stage of decomposition, which is considered the second level of decomposition (autolysis); and then active decay as the early third stage. In these stages, there was significantly less attraction and a smaller number of Calliphoridae. In the last stages of decomposition (advanced

decay and skeletonisation), there were no recorded numbers of this family of fly.

Figure 6: The relationship between temperature, humidity, and the population density of Calliphoridae in a year, over different seasons in Makkah city.

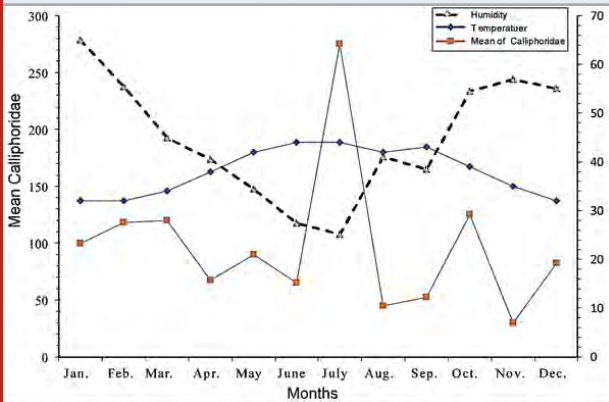
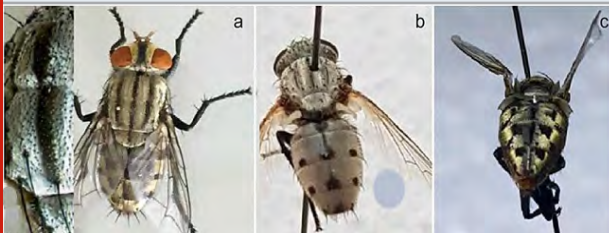


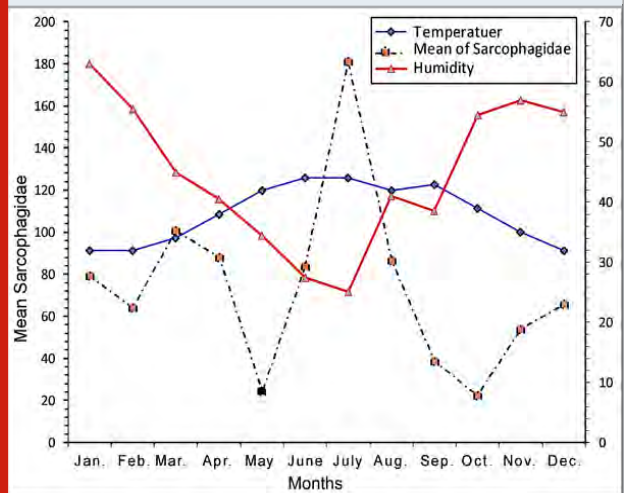
Figure 7: Classification of morphological characteristics, depending on keys of taxonomic descriptions under a digital stereo microscope; A: the appearance of three black lines clearly on the dorsal surface of the thorax from front to back in all species belonging to Sarcophagidae; B: the presence of dark spots as circle-like on both lateral pleura of the abdominal region, as well as triangular marks along the midline of the abdominal region in *W. bella*; C: large, silver, and cylindrical in shape, with some grey squares, which alternate with the silver colour to give the impression of a chess board in *S. dux*.



However, there were fluctuations and significant differences between their population densities. These results are in agreement with Al-Jameeli's (2021) study of the seasonal activity of *Ch. albiceps* showed that it was found continuously over the study period and this species is considered the dominant species. The species causes myiasis infection in its immature stages (larvae), occurring in tropical and subtropical areas, where they deposit their eggs on or near a wound or sore and, after hatching, the larvae burrow into the skin. The distribution and reproduction of *Ch. albiceps* flies have been observed in a variety of environments in terms of temperature and proximity to and distance above sea level (Al-Jameeli, 2021). *Ch. albiceps* is prevalent in the city of Riyadh, which is in the central region of Saudi Arabia (Al-Ahmed et al., 2006). In previous research, three and two peaks for *Ch. albiceps* activity have been recorded in Makkah and Jeddah over a year, respectively (Al-Ahmed et al., 2006).

Sarcophagidae family: The data showed that there were two species of Sarcophagidae, *Wohlfahrtia bella* (Macquart, 1836) and *Sarcophaga dux* (Thomson, 1869), which were important for forensic science in Makkah. These were classified using their morphological characteristics, depending on keys of taxonomic descriptions (Borror et al., 1981) under a digital stereo microscope. It has been noted that three black lines appear clearly on the dorsal surface of the thorax from front to back in all species belonging to this family (Fig. 7A). In addition, this species is distinguished by three clear features on the dorsal surface of the abdomen, the first being the presence of dark spots as circle-like on both lateral pleura of the abdominal region, as well as triangular marks along the midline of the abdominal region (Fig. 7B). The last feature relates to the size, shape, and colour of the abdomen, which is large, silver, and cylindrical in shape, with some grey squares, which alternate with the silver colour to give the impression of a chess board (Fig. 7C).

Figure 8: The relationship between temperature, humidity, and the population density of Sarcophagidae in a year, over different seasons in Makkah.

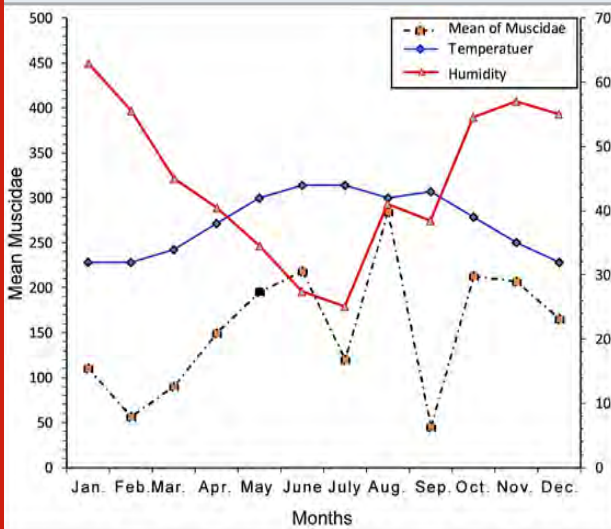


In the present study, two species of *W. bella* and *S. dux*, which belong to the Sarcophagidae family, were recorded in Makkah. This is the first recording of the *W. bella* species in Saudi Arabia, whereas the *S. dux* species have previously been observed and recorded in Riyadh, Saudi Arabia (Al-Misned et al., 2001). The Sarcophagidae family was shown to have a significant fluctuation in population density, reaching two peaks during the year in Makkah. These findings are consistent with many studies and past research conducted on Sarcophagidae (Al-Ghamdi et al., 2013; Al-Ghamdi et al., 2015). These two species, *W. bella* and *S. dux*, have also been found continuously in the city of Jeddah over a year (Algamdi, 2019). The findings reveal that the mean number of *W. bella* was $141.22 \pm 0.91SE$ and it was the most abundant and prevalent throughout the experiment time, with a 92% SD. The mean number of *S. dux* was $12.28 \pm 0.25SE$, at around 8% SD, making it less prevalent.

Figure 9: Classification of morphological characteristics, depending on keys of taxonomic descriptions under a digital stereo microscope; A: appearance of four dark stripes of equal length on the dorsal of the thorax; B: the first media M1 and the second media M2 NOT reached the edge of the apical margin; C: a pair of antennae that hold a long spine called an arista, which has long hairs on both sides; D: the terminal segment of the leg has a pretarsus that bears two claws; beneath these claws are two pads, called pulvilli, between which is an elongated spine called an empodium.



Figure 10: The relationship between temperature, humidity, and the population density of Muscidae in a year, over different seasons in Makkah city.



The results show that these species appeared continuously and sequentially on the cadaver throughout the year, with significant differences in population density (Table 1). In addition, the study recorded two peaks of activity for Sarcophagidae in the city of Makkah: a small peak in March and a high peak in July of the year 2019. The means of the highest peak and second peak were a population density of 181.3 ± 0.80 in July and 100.7 ± 1.50 in March, respectively (Fig. 8). The mean of the lower population density was

22.50 ± 0.90 in August, with significant differences between the population density over 2019 (Table 1). There was a negative correlation, though not a significant one, between the population density and temperature for Sarcophagidae ($R = -0.136$; $P = 0.52$), while the correlation with humidity was positive but not significant ($R = +0.181$; $P = 0.397$).

In regard to seasonal activity, the results showed a negative correlation and no significant difference between the seasonal abundance of Sarcophagidae and the temperature in Makkah. By contrast, in a study by Algamdi (2019), there was a positive correlation between seasonal abundance and temperature in Jeddah. These differences may be because of the geographical variance in the study areas. Over a year, between 10 and 12 generations of flies of diverse species have been recorded in warm regions, whilst in cold regions, reproduction is limited to only the warm months, though numerous species of fly have a period of diapause until conditions improve (Al Thabiani et al., 2016).

In general, there are several related factors and reasons that contribute to the increase in the population density and communities of various species of Sarcophagidae in Jeddah; some of these factors relate to location and climatic conditions, and others relate to certain habits, attitudes, and a low level of hygiene in poor neighbourhoods, as well as occasions when meat and sacrifices were common, resulting in increased organic and animal waste. This leads to the conclusion that the continued appearance of some species of Sarcophagidae in Jeddah is due to the spread of organic materials and blood and the low level of hygiene (Al-Solami, 2004).

Muscidae family: Within this family, one species was recorded, named *Musca domestica* (Linnaeus, 1758). Its average size is between 6 mm and 7.5 mm and it is dark grey in colour with four dark stripes of equal length on the dorsal of the thorax (Fig. 9A). In regard to the media veins (M), the first media M1 and the second media M2 have not reached the edge of the apical margin (Fig. 9B). The head is roughly spherical in shape and bears a pair of antennae that hold a long spine called an arista, which has long hairs on both sides (Fig. 9C). The terminal segment of the leg has a pretarsus that bears two claws; beneath these claws are two pads, called pulvilli, between which is an elongated spine called an empodium (Fig. 9D). The pads typically contain glandular capillaries ending with hairs that secrete an oily substance that causes the tips of the hairs to adhere to inverted surfaces. These structures and this substance provide the traction and stickiness that allows the insects to climb smooth or horizontal surfaces.

The monthly fluctuations in the population dynamics of *M. domestica* over a year in Makkah were recorded. Table (1) and Figure (10) show the fluctuations in the population dynamics with the changes in seasonal factors, specifically humidity and temperature. The mean population density was 285.3 ± 0.35 in August, the highest by a significant margin, compared with the mean population density in September, which was the lowest at 45.30 ± 0.22 . The mean of the highest abundance of *M. domestica* was 110 ± 0.50 in January, which decreased to 56.80 ± 0.15 in February.

This decrease was followed by a continuous increase in the abundance of *M. domestica* to reach a small peak in June; the mean population density of the small peak was 218.8 ± 0.50 , which then declined to 120.3 ± 0.13 in July. After this decline, the mean population density of *M. domestica* reached its highest peak in August, which was followed by simple fluctuation in population density from September to December 2019.

Table 1. The monthly abundance of flies' species of forensic importance in Makkah, Saudi Arabia (Jan.-Des.2019)

Months	Mean*±SE		
	Muscidae	Sarcophagidae	Calliphoridae
1	110.8±0.50 i	79.25±0.50 d	99.75±0.11 c
2	56.80±0.15 k	64.25±0.15 e	118.0±0.25 b
3	90.00±0.25 j	100.7±1.50 b	120.0±0.25 b
4	149.8±0.75 g	88.25±1.25 c	67.50±0.75 e
5	195.3±0.12 e	24.25±0.25 h	90.00±0.50 cd
6	218.8±0.50 b	83.75±0.50 c	65.00±0.26 e
7	120.3±0.13 h	181.3±0.80 a	275.0±0.27 a
8	285.3±0.35 a	86.75±0.25 c	45.00±0.28 f
9	45.30±0.22 l	38.75±0.30 g	52.50±0.15 f
10	213.3±1.50 c	22.50±0.90 h	125.0±0.11 b
11	206.8±0.50 d	53.75±0.35 f	30.00±0.25 g
12	165.3±0.16 f	65.75±0.50 e	82.50±0.50 d
LSD	3.23	5.38	3.00
P	0.0001	0.0001	0.0001

* Means followed by the same letter are not significantly different according to LSD

The results showed that the abundance of *M. domestica* rose gradually with progressive increases in temperature over the months from February to July. However, there was a positive, but not significant, correlation between the population density and temperature for Muscidae ($R=0.52$; $P=0.65$). In regard to the effect of humidity, it was recorded that the population of *M. domestica* increased gradually with decreasing humidity from February to June. Thus, there was a negative correlation between the population density and humidity, but not a significant one ($R=-0.05$; $P=0.78$).

The study showed that adult *M. domestica* was present throughout the year, but at different densities depending on the prevailing climatic conditions. The seasonal activity of *M. domestica* Makkah City almost similar to observation of many researchers in Kingdom of Saudi Arabia (Al-Shareef, 2016; Alikhan et al., 2018; AL-Ghamdi, 2019).

CONCLUSION

In this study, forensic insects in Makkah were identified using the morphological characteristics of adults, so we recommend the identification of insects at various stages of their life cycle, such as eggs, larvae, and adults using

molecular identification techniques.

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Normative Reference Value for Stereognosis Among Adult Population: A Cross-Sectional Pilot Study

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ABSTRACT

Processing of sensory input entails recognizing, categorizing, and interpreting data to generate an adaptive reaction. The sensory sub-types include barognosis, stereognosis, graphesthesia, two-point discrimination, and tactile localization. Stereognosis is the capacity to recognise the form and shape of a three-dimensional item and, consequently, its identity, by tactile manipulation of that object in the absence of visual and aural inputs. Therefore, manual stereognosis is necessary for integrating all hand sensations. So, the aim of this study is to determine the normal reaction time of manual stereognosis in adults. A pilot cross-sectional study was done on age group of 18-40 years. The stereognosis test was performed first on dominant hand and then on non-dominant hand using 5 objects (key, coin, safety pin, comb, pencil) with participant's eyes closed during the test. The time taken to identify the objects was recorded using stopwatch. Then, mean normal reaction time was calculated. Student t-test was used to compare any differences between right & left-hand side stereognosis and between male & female gender. Results from the study show normal mean values of reaction time in seconds was 3.04 for right hand and 2.09 for left hand in adults. Data also shows that there were statistically significant differences between right & left-hand side stereognosis. However, no differences were found between male & female stereognosis using independent t-test. These normative results of data will serve as a useful, simple, reproducible, rapid assessment of stereognosis and also aid in planning of treatment.

KEY WORDS: ADULTS, HAND SKILLS, NORMATIVE VALUES, STEREOGNOSIS, SENSORY INTEGRATION.

INTRODUCTION

Processing of sensory input entails recognizing, categorizing, and interpreting data to generate an adaptive reaction. Kilroy (2019) Exteroceptive and interoceptive functions of somatosensory system govern how we perceive and react to stimuli coming from outside and inside the body, respectively. Abaira (2013) There are 3 categories of sensations i.e., superficial feeling, deep experience and integrated cortical sensations. The sensory sub-types include barognosis, stereognosis, graphesthesia, two-point discrimination, and tactile localization, Spillane (2013). Stereognosis is a complex and multi-faceted evaluation that

is typically included in the sensory examination. Carlson (2009) Stereognosis is the ability to recognise the form and shape of a three-dimensional item and, consequently, its identity, by tactile manipulation of that object in the absence of visual and aural inputs, Fujii (2015).

In order to obtain discriminative touch and proprioceptive information, manual stereognosis requires healthy peripheral sensory pathways, specifically the dorsal column-medial lemniscus tract (DCMLT). Receiving this information is necessary but insufficient for stereognosis because parietal lobe cortex's processing centres must be operational as well. The deterioration of this ability can be detrimental, especially in tasks requiring fine motor skills, as impaired

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stereognosis is linked to decreased manual dexterity in people, Schermann (2021).

Therefore, manual stereognosis is necessary for integrating all hand sensations. Stroke is commonly the cause of manual stereognosis impairment, which has a debilitating effect. Fujii (2015) According to Reitan and Wolfson, stereognosis varies between individuals with brain injury and their unaffected peers. Reitan (2002) Other researchers have reported stereognosis impairments to differing degrees (42% to 97%) and have pushed for adding stereognosis ability as a crucial evaluation factor. Goldner (1966) & Van Heest (1993) Because the hand cannot receive and perceive information from the environment, decreased stereognosis is considered to predict limited functional capacity. Carlson (2009) Many assessment methods employ stereognosis as a measure of the upper extremity's functional potential, with lower stereognosis suggesting a lower possibility of functional improvements following surgery, Goldner (1966), Van Heest (1993), Zancolli (1983), Hoffer (1988), Thometz (1988).

To compare the value of stereognosis in affected people with normal value, main focus of the study is to determine the normal reaction time of manual stereognosis in adults. These normative results of data will serve as useful, simple, reproducible, rapid assessment of stereognosis and also aid the planning of treatment. Normative value of stereognosis is important to check hand sensory functions. There was no standard normative reference value available for stereognosis among adults.

Hence to fill this gap in the knowledge, the need of this study was to establish the normative value of stereognosis among adults of age 20-40 years. This study will also help

to recognise abnormal sensory function of hand in diseased individuals by comparing it with normative values of stereognosis in normal individuals. The aim of the present study is to find the normative reference value of stereognosis in adults. The objective was to compare normative reference value of stereognosis between right and left hand side and male & female participants.

MATERIAL AND METHODS

This study was a pilot cross-sectional study conducted at College of Physiotherapy, PGIMS Rohtak. This study was done on 40 adults individuals with age group 20-40 years in which 16 males and 24 females were included. There was 3 people with left hand dominance and 37 with right hand dominance. Convenient sampling method was used to recruit the subjects. Duration of study was 2 months. Inclusion criteria: Both male and female participants who were willing to participate in the study were selected with age group of 20-40 years. Exclusion criteria: Participants with neurological illness, any congenital deformity of hand, with recent hand injuries and fractures, with psychological condition that interferes with the test were excluded from the study. Materials used: Paper, Table, Chair, Pen, Stopwatch, Key, Pencil, Comb, Coin, Safety pin.

The procedure of stereognosis was explained to the participants. Participants were sitting in comfortable position with eyes closed. The stereognosis test included 5 daily used objects i.e., key, coin, pencil, comb and safety pin. Each object was placed separately first in dominant hand and then in non-dominant hand. The time taken to identify the objects was recorded using stopwatch. The test was repeated 3 times to obtain an average value and 1 min rest was given between each reading, Kinnucan (2010).

Table 1. Comparison of right and left hand side stereognosis using independent t-test

Stereognosis	Right		Left		t-value	p-value
	Mean	SD	Mean	SD		
Key	3.17	2.74	2.15	0.64	2.30	0.02*
Coin	2.59	0.89	2.07	0.67	2.95	0.00*
Pencil	3.05	1.50	2.24	0.92	2.90	0.01*
Comb	2.85	1.31	1.92	0.77	3.88	0.00*
Safety pin	3.56	1.85	2.06	0.73	4.80	0.00*

* Significant difference at 0.05 level

The SPSS statistical package (version 25.0) was used to analyse the data. Descriptive statistics was used to calculate the mean and standard deviation of time taken to identify various objects. Student independent t-test was used to find any difference between right- & left-hand side and gender difference between male & females. For all statistical tests,

a p-value ≤ 0.05 was taken as a significant difference.

RESULTS AND DISCUSSION

There were 16 males with mean age 27.48 ± 5.35 years and 24 females with mean age 26.76 ± 4.84 years. Table 1 shows

mean±SD value of stereognosis with various objects for right & left hand side and also shows that there was statistically significant difference in stereognosis between right & left hand side with various objects i.e., key, coin, pencil, comb and safety pin ($p \leq 0.05$). Table 2 shows mean±SD value of

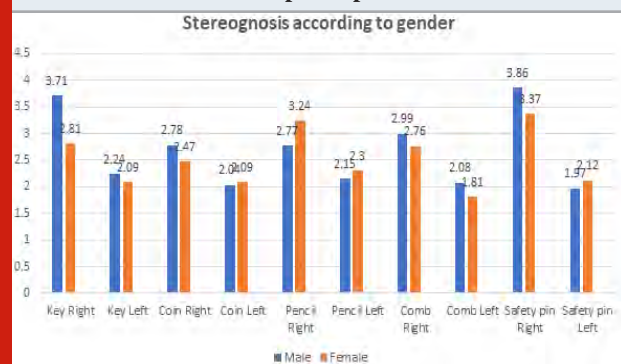
stereognosis using various objects in both male & female and no statistically significant difference between male and female stereognosis with various objects i.e., key, coin, pencil, comb and safety pin ($p \geq 0.05$). Figure 1 represents bar graph showing comparison of stereognosis mean value between male and female participants.

Table 2. Comparison of male and female stereognosis using independent t-test

Stereognosis		Male (16)		Female (24)		t-value	p-value
		Mean	SD	Mean	SD		
Key	Right	3.71	4.18	2.81	0.98	1.08	0.32
	Left	2.24	0.81	2.09	0.50	0.71	0.48
Coin	Right	2.78	1.21	2.47	0.59	1.09	0.28
	Left	2.04	0.88	2.09	0.50	0.26	0.80
Pencil	Right	2.77	1.65	3.24	1.40	0.96	0.34
	Left	2.15	0.89	2.30	0.95	0.50	0.62
Comb	Right	2.99	1.55	2.76	1.15	0.50	0.60
	Left	2.08	1.10	1.81	0.44	1.07	0.29
Safety pin	Right	3.86	2.52	3.37	1.23	0.83	0.41
	Left	1.97	0.95	2.12	0.56	0.61	0.55

*Significant difference at 0.05 level

Figure 1: Showing comparison of stereognosis mean value between male and female participants



The normative value of stereognosis is not present in the existing data. There is dearth of study on stereognosis. Hence this study was done to find the normative value of stereognosis in adult population. Previous studies have been conducted on subjects with carpal tunnel syndrome, Hsu (2014) cerebral palsy, Dahlin (1988) comparison of oral versus manual stereognosis, such as of Fujii (2015) in stroke patients and of Uma Maheswari (2021) worked after hand surgery and stereognosis in older subjects.

This study was done on a population of 40 adults with age group of 20-40 years. Three trials were done with eyes closed and average of 3 trials was taken to obtain the normative value for stereognosis. The present study confirmed that

there was significant difference between dominant and non-dominant side of hand performance of stereognosis. Mean values show that dominant hand i.e., right hand in mostly individuals has taken more time in recognising objects as compare to non-dominant hand i.e., left hand. This may be due to maturation effect as the test was first performed on dominant side than on non-dominant side.

Results show that there were no significant differences between male & female stereognosis mean values. Average reaction time to recognise objects in females was less as compare to males but it fails to reach statistically significance level. So, females have better stereognosis as compare to males. These results are consistent with a previous study by Dhanalakshmi et al. (2019) in which they found hand dexterity function was performed faster by females as compare to males.

CONCLUSION

Normative value of stereognosis is important to know for comparing it with any disease. Normative value of right hand is 3.04 sec. and of left hand is 2.09 sec. in age group of 20-40 years. Normative value of males & females in right hand are 3.11 sec. & 3.04 sec. respectively and in left hand are 2.08 sec. & 2.08 sec. respectively. These reference normative values can be used for the assessment of stereognosis and can also be used to compare with any disease so that treatment can be planned accordingly.

Future recommendations: The future study should include large sample size with equal numbers of male and female participants for better efficacy of result. Number of objects used for stereognosis should be more.

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Phylogeny of Strains of Tomato Leaf Curl Virus from Agroclimatic Zones of Gujarat

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ABSTRACT

With an increasing rate of global warming and unstable climatic conditions concerns with regards to epidemiology of plant viruses are on the rise. Studies suggest accelerating climatic changes shall severely affect the management of pest and diseases in cultivated crops. Tomato Leaf Curl Virus (TLCV) is an economically affecting viral infections of tomato (*Lycopersicon esculentum*). The disease causes severe yield loss and major economic impairment. The current study was therefore taken up to understand the influence of agro climatic zones on diversity of TLCV in Tomato plants. Samples of TLCV infected Tomato plants exhibiting varying symptoms were collected from seven different agro climatic zones of Gujarat followed by isolation of viral particles, molecular characterization and development of phylogenetic tree. Interestingly the molecular analysis of the isolated viral samples indicated little influence of climatic conditions on the types of TLCV infecting the tomato plants.

KEY WORDS: BEGOMOVIRUS, LYCOPERSICUM ESCULENTUM, GUJARAT, SOLANACEOUS, TLCV.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is an essential solanaceous crop cultivated worldwide. After China, India is the highest tomato producer of the world. Gujarat has a second highest productivity after Karnataka. Viral infections are the major cause of economically devastating diseases in tomato. Of these Begomovirus is the most widespread and deeply studied genera of plant virus comprising 162 known species infecting tomato. Begomoviruses are monopartite or bipartite, whitefly-transmitted geminiviruses that are found in the Eastern (both genome types) and Western Hemisphere (only bipartite are thought to be endemic. The acceleration in tomato virus discovery is far superior to the post discovery characterization. This lag leads to continued economic damages from known virus as well. (Shankar 2021, Rivarez et al. 2021, Bozbuga et al 2022).

Tomato Leaf Curl Virus (TLCV) is one such economically affecting viral infections of tomato. The disease causes symptoms such as leaf lamina yellowing, upward curling and distortion of leaf, decrease in size of new leaves, wrinkled appearance of leaf, decrease in internodes, reduction in height of plants, and flower drop from plant prior to fruiting. All this occurs within 2 to 3 weeks after infection. The

disease was first identified in Israel. In Indian subcontinent, Tomato Leaf Curl Disease (TLCD) is a plays a major role in reduced crop productivity for tomato cultivators with numerous reports on new strains being documented (Rivarez et al. 2021). Tomato Leaf Curl Virus (TLCV) is a viral disease, transmitted by vector whitefly. TLCV belongs to geminiviridae family and has a small geminate particle of 20×30 nm. Genome of TLCV consists of both mono- and bipartite genomes encapsulated by single capsid protein. Although some Indian TLCV isolates such as tomato leaf curl New Delhi virus (TLCNDV) and tomato leaf curl Palampur virus (TLCPaV) are bipartite (DNA-A and DNA-B) in nature, most of the TLCV isolates reported to date have monopartite (DNA-A) genome organization. These are single stranded DNA genomes having a size of approximately 2.7 kb size. They code for viral factors needed for viral replication, transmission, encapsidation and spread (Reddy et al. 2005; Wendy et.al. 2020, Sohrab et. al. 2021).

Current global environmental concerns such as unpredictable climatic conditions and global warming increases probabilities of poor management of pest and diseases in the cultivated crops (Trebicki P. 2020). In order to facilitate virus disease forecasting and prevention of viral disease outbreak in tomato it is important to understand the epidemiology of these viruses. The current study therefore focuses on the molecular characterization of TLCV to understand the

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influence of agro climatic zones on its diversity in Tomato plants.

MATERIAL AND METHODS

Sample collection: Samples of TLCV infected Tomato plants exhibiting varying symptoms were collected from seven different agro climatic zones of Gujarat (Indian Horticulture Database., 2011). The virus were classified into 11 strains based on the symptoms and geographical location of collection site.

Isolation of viral samples: The collected leaf samples were washed with water and were stored in -20 °C deep freezer. 100 grams of leaves were homogenized using mortar and pestle in 0.1 M Phosphate buffer. The suspension was then filtered through muslin cloth. The filtrate was collected and stirred at 4°C with half volume of Chloroform. After 30 minutes the mixture was centrifuged at 10000 rpm for 15 minutes. After centrifugation, PEG and NaCl were added to the supernatant while stirring at 4°C. After 2 hours for 60 minutes centrifugation of the mixture was done at 16000 rpm. The precipitates were dissolved in 100µl of 0.1 M Potassium Phosphate buffer, pH 7.8. This was filtered using 0.45µm Syringe filter (Palmer et al.,1998). To confirm the

isolation of viral particles, the samples were characterized by Transmission Electron Microscopy (TEM).

DNA extraction: DNA extraction was done for the isolated viral samples using TempliPhi kit, GE Healthcare, U.S.A. Rolling circle amplification (RCA) was used to amplify the DNA. The obtained DNA was digested using Kpn I. The resultant fragment (~2.8-kb) was cloned in pUC18 plasmid. The full genome sequence was determined for all the viral samples obtained.

Phylogenetic Analysis: The nucleotide sequences obtained in this study were deposited in GenBank. Sequences were retrieved and a phylogenetic tree of all the 11 samples along with one outgroup of TLCV retrieved from NCBI database was constructed using CLUSTAL W program (random seed number, 111; bootstrap value, 1000).

RESULTS AND DISCUSSION

Collection of samples from different agroclimatic regions Twenty six samples of tomato leaves infected with symptoms similar to TLCV were collected from different agroclimatic regions of Gujarat. The viral samples were classified into 11 strains based on the symptoms observed and geographical location of collection site (Table I).

Table 1. Types of virus found in different agro climatic zones

Code	Agro climatic zones	Sample collection region
GJ 1	South Gujarat Heavy rainfall Zone	Valsad
GJ 2	South Gujarat Zone	Surat, Ankleshwar
GJ 3	Middle Gujarat Zone	Balashinor, Matar, Kheda
GJ 4	North-West Zone	Vijapur, Himatnagar
GJ 5	North Saurashtra Zone	Botad
GJ 6	South Saurashtra Zone	Kodinar, Jetpur
GJ 7	Bhal and Costal Zone	Dholka

Isolation of viral samples: The virus were isolated and confirmed to be TLCV by TEM (Figure I).

Figure 1: Electron Microscopic image of the Tomato Leaf Curl Viral particles



Molecular Analysis: Total 11 samples were sent for molecular analysis and the sequencing was done by a third

party institution. The sequences were submitted and the Genbank ID was procured. The details were given in the Table II.

Phylogenetic Tree: The phylogenetic tree was developed from the molecular study of the different samples of TLCV collected from the different agro climatic zones of Gujarat (Figure II).

DISCUSSION

The need to surpass food production over population explosion not only demands increasing production but also decreasing yield loss (Jones et.al. 2012). During the current times of climatic instability, studying the influence of climatic alterations on the prevalence of disease in wild type and cultivated plants is therefore of paramount importance. Viruses cause almost half of the evolving plant diseases worldwide (Jones 2019). Despite all investigations in viral diseases control, no antiviral products are available for plant

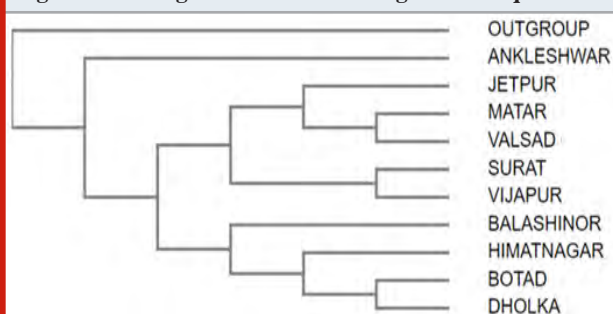
disease management till now (Petrov et.al. 2021). In the past decade 45 novel species of virus infecting tomato have been identified (Rivarez et al. 2021). Tomato leaf curl disease (TLCD) is the most common viral disease in the tomato plant (Arooj et.al. 2017). In order to facilitate virus disease

forecasting and enhance TLCV management, it is vital to understand the epidemiology of the disease. The current study therefore focused on, the molecular characterization of TLCV from different agro climatic zones of Gujarat with special attention on understanding the climatic influence on its diversity in Tomato plants.

Table 2. GenBank Accession Numbers for sequenced genomes

Sr. No.	Area	GenBank Accession Number		
			DNA-A	DNA-B
1	Ankleshwar	MT551610	MT316381	MT295294
2	Balashinor	MT551611	MT316382	MT295295
3	Botad	MT551612	MT316383	MT295296
4	Dholka	MT551613	MT316384	MT295297
5	Himatnagar	MT551614	MT316385	MT295298
6	Jetpur	MT551615	MT316386	MT295299
7	Kodinar	MT551616	MT316387	MT295300
8	Matar	MT551617	MT316388	MT295301
9	Surat	MT551618	MT316389	MT295302
10	Valsad	MT551620	MT316390	MT263149
11	Vijapur	MT551621	MT316391	MT295303

Figure 2: Cladogram of the obtained genomic sequences



Several previous reports suggest temperature to affect susceptibility and symptom development of other viral diseases (Llamas-Llamas et al. 1998; Zhang et al. 2012; Prasch et al. 2013; Zhong et al. 2013; Ghoshal et al. 2014; Patil et al. 2015). These studies indicate the one type of infection under specific climatic conditions should predominate other infections. From our results it was interesting to observe that the sequences of viruses obtained from the same agro climatic zones were genetically very different. The viruses were separated into two main clads one consisted of Dholka, Botad, Himatnagar and Balashinor. Each of these isolates were from different agro climatic zone. The second clad had viruses from all agro climatic zones. No distinct pattern of viral infection based on agroclimatic zone was observed. Virus isolated from infected plants of Ankleshwar, South Gujarat Zone showed totally distinct genetic makeup.

Although previously we have reported severity of TLCV infection to influenced by climatic factors (Shelat et al.,2014), the current study confirms the type of TLCV strain

not to be influenced by the climatic conditions. We therefore conclude little influence of climatic parameters on the types of TLCV strain infecting the tomato plants.

A conflict of Interest: No conflict of interests.

Data Availability Statement: All data / results / information is available with the authors and can be shared on a reasonable request made to the corresponding author when required.

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Solid State Fermentation of Groundnut Shell By *Schizophyllum commune* BCC26414 For Production of Cellobiose Dehydrogenase

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ABSTRACT

Cellobiose dehydrogenase (CDH) enzyme is secreted extracellularly by wood-rotting fungi of the phyla Basidiomycetes and Ascomycetes. The reducing ends of cellobiose, lactose, and maltose are oxidized by CDH to produce their respective lactones. These lactones are consequently converted into their carboxylic acids such as cellobionic acid, lactobionic acid, and maltobionic acid. Due to its commercial unavailability and its applications in various fields, there is a need for cost-effective CDH production. In the present work, *Schizophyllum commune* BCC26414 has been used for CDH production by solid-state fermentation (SSF). CDH production was optimized by one factor at a time (OFAT) approach in terms of initial moisture content, inoculum size, incubation temperature, particle size, and fermentation time. BBD (Box-Behnken Design) was used to perform statistical optimization of CDH production using statistical software, Response Surface Methodology (RSM). Maximum CDH production was obtained when groundnut shell was used as a substrate at 30°C on 9th day of incubation, with 0.5mm to 1mm particle size, 2 ml inoculum size, and the initial moisture content 50% using *Schizophyllum commune* BCC26414. RSM enhances enzyme production to 1.6-fold as compared to unoptimized conditions. This is the first report on solid-state CDH production using groundnut shells as solid substrate. A variety of CDH applications have been reported in the fields of biomedical, biocatalysts, bioremediation, and biosensors. This study will be helpful in the cost-effective production of CDH for various applications.

KEY WORDS: BOX-BEHNKEN DESIGN (BBD), CELLOBIOSE DEHYDROGENASE, GROUNDNUT SHELL, RESPONSE SURFACE METHODOLOGY (RSM), *SCHIZOPHYLLUM COMMUNE* BCC26414, SOLID-STATE FERMENTATION (SSF).

INTRODUCTION

Fungi are the most important and well-known group of microorganisms, having vast potential to degrade wood components such as cellulose, hemicellulose and lignin by the action of enzymes (Henriksson et al. 2000). One of these enzymes is Cellobiose dehydrogenase [E. C. 1.1.99.18], which is reported in several wood-decaying fungi belonging to the phylum basidiomycetes and ascomycetes (Henriksson et al. 2000; Banerjee et al. 2021). Cellobiose dehydrogenase (CDH) is mainly secreted by microbes including bacteria and fungi (Cameron et al., 2001). Disaccharides and oligosaccharides with β -1-4 glycosidic linkages are susceptible to oxidation of their reducing ends oxidized by CDH. These disaccharide and oligosaccharides produce their corresponding lactones and these lactones spontaneously convert to their carboxylic acids (Gangwar et al. 2021).

CDH is a monomeric protein and consist of two domains: a flavin domain that contains FAD and a heme domain that contains cytochrome b-type heme, which is connected by a serine and threonine-rich amino acid linker that is sensitive to protease. Catalytic and cellulose binding properties are present in FAD-containing fragment. Monosaccharides are the poor substrates for CDH due to lack of β -1-4 glycosidic linkages (Baminger et al. 2001; Gupta et al. 2014). CDH has important applications in many biotechnological fields such as pharmaceutical, cosmetics, food industries, and different clinical applications (Nyanhongo et al. 2017).

The solid-state fermentation (SSF) method can increase enzyme yield while minimizing the cost of enzyme production. The most frequent microorganisms utilized in SSF are filamentous fungi because they can grow on solid substrates with little water content (Mrudula and Murugammal, 2011). There are numerous studies describing the use of agro-industrial wastes as solid substrates in SSF, such as wheat straw (Li et al. 2008); while wheat bran, and

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rice- straw as substrates for submerged fermentation (Rai et al. 2020) for the production of CDH. SSF is reported to be especially suitable for fungi rather than bacteria (Patidar et al. 2018). The SSF process is also known to be the most effective method for producing enzymes due to its high productivity, straightforward approach, inexpensive capital investment, low energy need, low water output, higher product recovery, and lack of foam buildup (Mrudula and Murugammal, 2011). In SSF, fungi primarily use agro-industrial wastes as a medium for the synthesis of metabolites and enzymes. Fungi are more appropriate for use in SSF processes since they can grow and exploit agricultural waste as their natural environment. Fungi have been believed to be the organisms most adapted to SSF and can colonize solid substrates because their hypha can grow on particle surfaces and enter the inter-particle gaps (Patidar et al. 2018).

The CDH production by filamentous fungi under Submerged Fermentation has been studied extensively. However, there are very few reports on CDH production using SSF. In the present study, the organism was isolated and grown in SSF conditions to produce CDH by utilizing agro-industrial waste as a solid substrate. One factor at a time (OFAT) and Box-Behnken Design (BBD) of Response Surface Methodology (RSM) were used to statistically optimize the production.

MATERIAL AND METHODS

Isolation of fungi: Zip lock bags were used to collect samples from a variety of sources in Indore, India. The sources include air, damaged wood, soil, and municipal garbage. These samples were serially diluted, inoculated, and incubated on PDA media for 3 days at 28 °C. All isolates were preserved on PDA media for further screening.

Screening of potential CDH producer: The primary screening was carried out by growing isolates on Carboxymethyl cellulose (CMC) agar media containing (per liter) 10g CMC, 0.1g Yeast extract, 0.25g Peptone, 1.4g (NH₄)₂SO₄, 2g KH₂PO₄, 0.3g MgSO₄·7H₂O, 0.3g Urea, 0.3g CaCl₂, 3.34mg ZnSO₄·7H₂O, 5mg FeSO₄·7H₂O, 1.56mg MnSO₄·7H₂O, 2mg CoCl₂ and 15g Agar, pH 5.0. It was inoculated at 28 °C for 3 days (Shams et al. 2004). After incubation, 0.1% Congo red was flooded on agar media plates for 10 minutes and washed with 1 N NaCl. The fungi which formed a clear zone by flooding Congo red solution were selected and used for secondary screening (Amrinola et al. 2012).

The isolates selected from primary screening were grown in liquid media containing (per liter) 30g Microcrystalline cellulose, 30g Yeast extract, 1.0g MgSO₄·7H₂O and 0.3 ml trace element solution. Trace element solution contains (per liter), 0.3g MnCl₂·4H₂O, 3g H₃BO₃, 0.1g CuSO₄·5H₂O, 2g CoCl₂·6H₂O, 0.2g NiCl₂·6H₂O, 1g ZnSO₄·7H₂O and 4ml conc. H₂SO₄ (Ludwig et al. 2003; Fischer et al. 2014). The pH of the media was adjusted to 5. The cellulose-containing medium was prepared and divided into 100 ml aliquots in 250 ml Erlenmeyer flask and autoclaved at 121°C for 15 min. Each flask was inoculated with two agar plugs (6

mm diameter) taken from 5-day-old agar culture and was incubated at 150 rpm agitation condition at 30 °C for 1 to 14 days (Baminger et al. 2001). Fungal culture supernatant was separated by centrifugation at 8000 rpm for 20 min at 4 °C and used as a crude extract for enzyme assay.

Identification of fungal isolate: The fungal isolate W3 grown on PDA plates, was identified based on morphological and molecular characteristics. The growth characteristics and its microscopic observations were used to make a preliminary identification of the isolate. The molecular identification of the selected fungal isolate was done at the National Fungal Culture Collection of India (NFCCI), Pune, India. The Genomic DNA was isolated from W3 fungal isolate in pure form. The primers ITS4 & ITS5 were used for effective amplification of ITS-rDNA partial gene. The ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit was used to set up the sequencing PCR. The ABI 3100 automated DNA sequencer's raw sequence was checked manually for consistency and compared to 18 r DNA sequences using the BLAST tool (Altschul et al. 1990). Closely related sequences were aligned using Clustal W software, and a phylogenetic tree based on the neighbor-joining (NJ) approach was created using the MEGA X program (Kumar et al. 2016).

Production of CDH by SSF: Preparation of inoculum: *Schizophyllum commune* was point inoculated on PDA media plate (90 mm diameter) and incubated for five days. Mycelia were scrapped from cultured plate after addition of 5ml sterile distilled water. Mycelia were collected and suspended in 10ml sterile distilled water and then vortexed with few glass beads of 0.1 mm diameter. The above fragmented mycelial culture was used as the inoculum for CDH production (Saha et al. 2008; Petrikkou et al. 2001).

Substrate selection: Agro-industrial residues mainly orange peel, wheat straw, banana peel, sugarcane bagasse, soybean straw, pineapple leaf, orange pulp, corn cob, groundnut shell, wood dust, coconut husk, and parthenium grass were screened for CDH production by SSF. These substrates were dried and crushed. SSF was performed using mineral solutions and distilled water to maintain a moisture content of 80% (v/w) while using 100 g of dry substrate in a 500 ml Erlenmeyer flask. The mineral solution contains (per liter) 26g KCl, 26g MgSO₄, 76 g KH₂PO₄ and 0.3 ml trace element solution (as mentioned above in secondary screening) (Abdullah et al. 2016). All the flasks were autoclaved for 20 minutes at 121 °C. 1ml of inoculum was added to each flask and incubated at static condition at 30 °C for 1 to 14 days.

Enzyme extraction: The fermented medium (1g) was completely mixed with 100 mM sodium acetate buffer, pH 4.5 (1:15 w/v) and incubated at 30°C on a shaker at 120 rpm for 1 hr. The mixture was centrifuged at 5000xg for 15 min. at 4°C after being filtered using Whatman no. 1 filter paper. The enzyme assay was performed using the supernatant as a crude enzyme.

Enzyme assay and protein estimation: The reduction in absorbance of Dichloro Phenol Indo phenol (DCPIP) was

used to determine CDH activity. The CDH activity assay was carried out by taking the reaction mixture containing 100 μ l of 3mM DCPIP (in 10% ethanol), 100 μ l of 300mM lactose and 20 μ l of 80mM NaF in 100mM sodium acetate buffer (pH 4.5). The reaction was initiated by adding 100 μ l of crude CDH. The decrease in absorbance at 520 nm was monitored for 10 minutes (Ludwig et al. 2003; Sulje et al. 2015). One unit of CDH activity has been defined as the amount of enzyme required for reduction of one μ mol of DCPIP per min, under standard conditions. The amount of total protein was determined using the Folin Lowry method with bovine serum albumin (BSA) as standard (Waterborge and Matthews, 1994). All the experiments were performed in triplicate.

Optimization of CDH production using OFAT approach:

OFAT method was used for the optimization of parameters such as initial moisture content (%), inoculum size (ml), temperature ($^{\circ}$ C), particle size (mm), and fermentation time (days). These parameters were the most effective variables representing a significant influence on CDH activity (Latifian et al. 2007; Darabzadeh et al. 2019).

Moisture content: The crushed groundnut shell was taken in Erlenmeyer flasks and moisture content was set in the range 30% to 90%, with interval of 10 % using the mineral solution and distilled water (1:1). All the flasks were inoculated with 1 ml inoculum and incubated at 30 $^{\circ}$ C. The crude enzyme was extracted after 8 days and enzyme assay was performed.

Inoculum size: The crushed groundnut shell was taken in Erlenmeyer flasks and inoculum size was set in the range 0.5 ml to 3 ml, with an interval of 0.5 ml. All flasks had 50% moisture content and were incubated at 30 $^{\circ}$ C. The crude enzyme was extracted after 8 days and enzyme assay was performed.

Incubation temperature: The groundnut shell was taken in Erlenmeyer flasks, inoculated with 2 ml inoculum and 50% moisture content, and incubated at different incubation temperatures ranging from 20 $^{\circ}$ C to 40 $^{\circ}$ C with intervals of 5 $^{\circ}$ C. The crude enzyme was extracted after 8 days and enzyme assay was performed in each sample.

Particle size: The groundnut shell of different particle size (0.5, 0.5-1 mm, 1-1.5 mm and >2.0 mm) were taken in Erlenmeyer flasks. At this step, moisture content was 50%, inoculum size 2ml, and incubation temperature 30 $^{\circ}$ C. The crude enzyme was extracted after 8 days and enzyme assay was performed.

Fermentation time: The prepared groundnut shell substrate was taken in Erlenmeyer flask and enzyme was extracted at different fermentation time ranging from 5 to 14 days. In this case, moisture content was 50%, inoculum 2ml, incubation temperature 30 $^{\circ}$ C and particle size 0.5mm to 1mm. The crude enzyme was extracted every 24 hours and enzyme assay was performed.

Table 1. Independent variables used in BBD experimental design

Variable	Name of parameters	Unit	Range and Levels		
			-1	0	+1
A	Fermentation time	Days	5	8	11
B	Moisture content	% (v/w)	30	50	70
C	Particle size of Groundnut shell	mm	0.5	1	1.5
D	Temperature	$^{\circ}$ C	25	30	35

Statistical Analysis: After carrying out all the experiments in triplicate, the mean values for each experiment were calculated. Analysis of variance (ANOVA) was used to examine the data, and a significant result was defined as a P value of less than 0.05 (Nghi et al. 2021).

Statistical optimization of CDH production using RSM: OFAT method was used for the optimization of parameters such as moisture content (%), inoculum size (ml), incubation temperature ($^{\circ}$ C), particle size (mm), and fermentation time (days). Out of these, four variables namely moisture content, incubation temperature, particle size, and fermentation time were identified to be critical parameters in fermentation method. Response surface approach with BBD was used to examine the relationship between these important parameters and their optimum level (Box and Behnken, 1960).

Design-Expert 11.0 software was used to set up the experimental range, and each independent variable was examined at three different levels (+1,0, -1) using BBD. (Table 1). The following equation was used for the number of experiments (N) required for the creation of BBD:

$$N = 2k(k-1) + C_0$$

Where, k denotes the number of variables and C_0 denotes the number of central points. By conducting 29 runs with five replicates at the centre point of experiment, this equation was used to construct a mathematical connection between four variables that are used in the production of CDH.

The following quadratic polynomial model equation was fitted using the response data:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

Where, Y stands for predicted response; β_0 for coefficient of fitted response; β_i for linear coefficient; X_i for independent variables; β_{ii} for quadratic coefficient; X_{ij} are variables interacting with each other and β_{ij} for interaction coefficient.

Table 2 denotes BBD's experimental design in coded levels for the four variables. 2ml inoculum was added for all the

experimental runs. The flasks were analysed for CDH production at regular time intervals, 5th, 8th and 11th day, as designed by BBD.

Validation of the experimental model: Under optimal conditions, the validation of the experimental model for CDH production was carried out in triplicates and the obtained findings were compared with the response predicted by the model.

Table 2. Process parameters for the production of CDH optimized using the BBD experimental design

Standard Order	Run Order	Fermentation time		Moisture content		Particle size		Temperature		Actual value	Predicted value
		Coded	Decoded	Coded	Decoded	Coded	Decoded	Coded	Decoded		
26	1	0	8	0	50	0	1	0	30	216.31	192.75
5	2	0	8	0	50	-1	0.5	-1	25	133.07	125.30
11	3	-1	5	0	50	0	1	+1	35	48.90	43.25
18	4	+1	11	0	50	-1	0.5	0	30	51.65	80.53
2	5	+1	11	-1	30	0	1	0	30	22.61	18.73
28	6	0	8	0	50	0	1	0	30	212.61	192.75
7	7	0	8	0	50	-1	0.5	+1	35	106.40	88.24
27	8	0	8	0	50	0	1	0	30	217.14	192.75
9	9	-1	5	0	50	0	1	-1	25	49.83	47.43
13	10	0	8	-1	30	-1	0.5	0	30	98.62	88.75
12	11	+1	11	0	50	0	1	+1	35	44.18	35.53
4	12	+1	11	+1	70	0	1	0	30	51.59	51.57
29	13	0	8	0	50	0	1	0	30	217.88	192.75
8	14	0	8	0	50	+1	1.5	+1	35	80.29	88.87
17	15	-1	5	0	50	-1	0.5	0	30	50.75	71.92
10	16	+1	11	0	50	0	1	-1	25	51.59	46.20
16	17	0	8	+1	70	+1	1.5	0	30	92.24	91.06
20	18	+1	11	0	50	+1	1.5	0	30	49.37	38.44
1	19	-1	5	-1	30	0	1	0	30	23.90	24.73
3	20	-1	5	+1	70	0	1	0	30	49.83	54.52
23	21	0	8	-1	30	0	1	+1	35	23.90	35.58
15	22	0	8	-1	30	+1	1.5	0	30	49.83	53.04
21	23	0	8	-1	30	0	1	-1	25	70.94	68.98
19	24	-1	5	0	50	+1	1.5	0	30	74.64	56.00
22	25	0	8	+1	70	0	1	-1	25	75.75	74.31
6	26	0	8	0	50	+1	1.5	-1	25	47.70	66.66
25	27	0	8	0	50	0	1	0	30	99.83	192.75
24	28	0	8	+1	70	0	1	+1	35	80.66	92.86
14	29	0	8	+1	70	-1	0.5	0	30	127.61	113.36

RESULTS AND DISCUSSION

Isolation and Screening of potential CDH producer: A total of 108 isolates were obtained from various sources such as soil, air, water, degraded wood, dung, compost, and infected plant parts. All isolates were primarily screened on CMC-agar plate and diameter of clear zone in each case was determined. Out of all isolates, 12 isolates hydrolyzed the CMC-agar plates and showed the zone of clearance in a range from 3.8mm to 20mm (Table 3).

The fungal isolates that produced a clear zone were selected for secondary screening. The secondary screening was used to confirm the CDH production in cellulose-containing liquid media under shaking conditions. W3 isolate showed maximum CDH activity. CDH activity was measured by a DCPIP-based assay. This fungal isolate was selected for further studies. Previous studies have reported that CDH is secreted by *Phanerochaete chrysosporium* (Bao et al. 1993), *Monilia* (Dekker et al. 1980), *Sclerotium rolfsii* (Sadana et al. 1988) and *Schizophyllum commune* (Fang et al. 1998).

The zone diameter does not accurately corresponds to CDH production because the media contains CMC, which is not the specific substrate for CDH production (Amrinola et al. 2012) and the zone may be because of some other

enzyme secretions. Similar results have been reported in *Cladosporium* isolates, where agar plate screening and liquid media cultivation were not parallel for CDH activity (Shams et al. 2004).

Table 3. Fungal isolates with zone of clearance of different diameter

Isolate name	Zone diameter (mm)	Isolate name	Zone diameter (mm)
E1	20.1±1.0	S10	9±2.2
G4	19.83±3.0	C2	8.1 ±1.5
B2	16.1 ±1.0	W6	7.1 ±1.5
C8	15.5±2.2	N1	6.8 ±3.0
W7	11.5 ±1.7	W3	5.1 ±0.5
C6	10.3±1.0	A2	3.8±0.5

Table 4. ANOVA table showing the experimental results using the Box-Behnken design.

Source	Sum of squares	Df	Mean square	F-value	p-value	
Model	85068.24	14	6076.30	6.01	0.0009	Significant
A-Fermentation time	60.12	1	60.12	0.0594	0.8109	
B-Moisture content	2941.57	1	2941.57	2.91	0.1103	
C-Particle size	2523.87	1	2523.87	2.49	0.1366	
D-Temperature	165.39	1	165.39	0.1635	0.6921	
AB	2.33	1	2.33	0.0023	0.9624	
AC	171.22	1	171.22	0.1692	0.6870	
AD	10.50	1	10.50	0.0104	0.9203	
BC	45.02	1	45.02	0.0445	0.8360	
BD	674.70	1	674.70	0.6668	0.4278	
CD	877.94	1	877.94	0.8677	0.3674	
A2	52656.29	1	52656.29	52.04	<0.0001	
B2	27632.78	1	27632.78	27.31	0.0001	
C2	10868.09	1	10868.09	10.74	0.0055	
D2	23004.58	1	23004.58	22.74	0.0003	
Residual	14165.06	14	1011.79			
Lack of Fit	3355.06	10	335.51	0.1241	0.9964	not significant
Pure Error	10810.01	4	2702.50			
Cor Total	99233.30	28				

Identification of fungal isolate: Depending on morphological and molecular characteristics, the isolated W3 fungal strain was identified. Morphological identification of W3 fungal strain has shown that the isolate is filamentous and makes white cottony mycelia on PDA plate (Figure 2). The BLAST results of the ITS sequence (Figure 3) obtained from NCBI indicate the relationship of *Schizophyllum commune* BCC26414 with other isolates. There are many reports in the literature which are related to the identification of fungi based on ITS sequence (Raja et al. 2017). The tested fungal strain showed 99.83 % sequence similarity with *Schizophyllum commune* BCC26414, whose accession number is FJ372690. Previously reported *Schizophyllum*

commune AS 5.391 also has the ability to produce CDH (Fang et al. 1998).

CDH production by Solid State Fermentation: Substrate selection: Several agro-waste materials were screened for CDH production using *Schizophyllum commune*. Out of these, six substrates (wheat straw, corn cob, groundnut shell, orange peel, soybean straw, and pineapple leaf) were utilized by the fungi in SSF for CDH production. Maximum CDH activity (133.3 U/ml) was obtained in groundnut shells and the least activity in wheat straw (15.25 U/ml) (Figure 4). According to Gupta et al. (2014), a cellulosic substrate with greater crystallinity may increase CDH production.

The cellulose of groundnut shell has a high degree of crystallinity index (Manrich et al. 2021) and wheat straw has a low crystallinity index (Liu et al. 2005).

Figure 1: Congo red is used as an indicator on an agar plate with CMC as the substrate. The zone of clearance surrounding the isolate revealed CMC degradation (a) control plate (b) W3 isolate

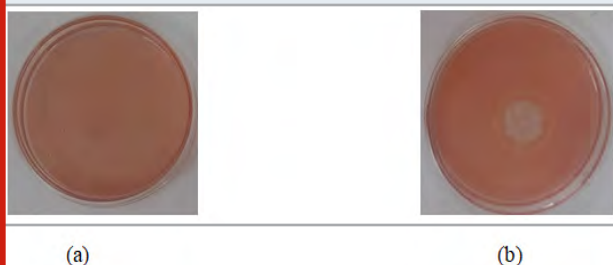
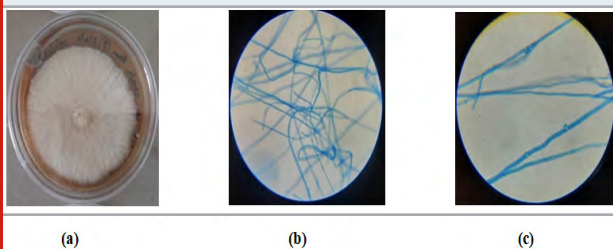


Figure 2: (a) W3 fungal isolate growth on potato dextrose agar. Microscopic view of *Schizophyllum commune* BCC26414 stained with lactophenol cotton blue at (b) 40 X (c) 100 X in oil immersion in bright field microscopy



Tramitomyces clypeatus grown in submerged fermentation on cellulose containing medium showed high CDH activity (55.88 U/ml) on eighth day at 30°C. *Tramitomyces clypeatus* produced the highest yield of CDH activity when compared to other organisms that also used submerged fermentation, including *Phanerochaete chrysosporium* (0.8 U/ml), *Schizophyllum commune* (0.15 U/ml), and *Sclerotium rolfsii* (7.5 U/ml) (Saha et al. 2008). Very few reports on CDH production using SSF are available. CDH production has been reported earlier from *Fusarium concolor* using wheat straw (Li et al. 2008) and *Coprinellus aureogranulatus* using rice straw as a solid substrate in SSF (Nghi et al. 2021). This is the first report of CDH production by SSF using groundnut shells as a substrate.

Optimization of CDH production using OFAT approach:

The one factor at a time (OFAT) method was used to optimise the growing conditions for the fungal isolate's production of CDH.

Moisture content: The fermentation medium must have a proper level of moisture since it influences microbial development and biosynthesis. As shown in Figure 5, highest enzyme production was obtained at 50% moisture content. Similar results were observed for protease production using wheat bran and rice bran as a substrate in SSF with 50% moisture content (Chutmanop et al. 2008). As reported in other studies, low moisture content decreases

nutrient solubility, water absorption, and substrate swelling, whereas excessive moisture content leads to the reduction of contact surface of fungus to the solid particles (Dutt and Kumar, 2014; Darabzadeh et al. 2019).

Inoculum size: An optimum inoculum size is required for enzyme production. As seen in Figure 6, the enzyme activity increased as the inoculum size increased from 0.5 ml to 2.0 ml. With further increase in inoculum size till 3.0 ml, the enzyme activity decreased. Similar results were reported by Gupta et al (2014), who found that increasing the inoculum size from 10% to 25% resulted in a decrease in CDH activity. This could be because at low inoculum size, a longer lag phase is required, which results in lesser CDH activity. Upon increasing the inoculum size, rapid growth, and hence more enzyme production is favoured. However, a comparatively large inoculum size decreases enzyme production due to the quick depletion of nutrients (Gupta et al. 2014).

Figure 3: A phylogenetic tree created using the neighbor-joining technique that shows the connections between isolated strains. Accession numbers from the NCBI database of each isolate are given in the tree



Temperature: The growth, physiology, and enzyme activity of microorganisms are strongly influenced by temperature. As shown in Figure 7, the enzyme activity increased as temperature increases from 20 °C to 30 °C. With further increase in temperature till 40 °C, enzyme activity decreased. Highest enzyme activity at 30 °C suggests its mesophilic nature also reported 30 °C as optimum production temperature for CDH activity in *Tramitomyces clypeatus* (Saha et al. 2008). This effect is due to the fact that at lower temperatures, substrate and product diffusion across the fungal cell is very less hence lowering the enzyme production. At elevated temperatures, the enzyme production declines due to its thermal denaturation (Dutt and Kumar, 2014; Lugani et al. 2015).

Particle size: Surface area is crucial for microbial adhesion and the mass transfer of different nutrients in SSF (Prakasham et al. 2006). As shown in Figure 8, the enzyme production increases upon increasing the particle size from 0.5mm to 1mm. A further increase in particle size till 2mm decreases the enzyme production. Similar results were observed by Matkawala et al (2019), where

1.4 mm particle size was considered optimum for alkaline protease production. An appropriate particle size provides an optimum surface area to facilitate microbial growth and product formation (Matkawala et al. 2019). Smaller substrate particles have more surface area, which promotes the development of microorganisms. However, too small substrate particles may cause substrate aggregation, which will slow the growth. Alternately, bigger particles size inhibits the growth of microorganisms because of their reduced surface area, lesser aeration, and mass transfer (Pandey et al. 2000).

Fermentation time: The fermentation time is associated with microbial growth and enzyme production. As shown in Figure 9, the enzyme activity increased up to 9th day, after which it decreased as observed till 14th day. Similar results have been reported in *Tramitomyces clypeatus*, where maximum CDH production was found on 8th day of submerged fermentation, at 30 °C under shaking conditions (Saha et al. 2008).

Figure 4: Substrate Selection for CDH production by *Schizophyllum commune*. The data are represented as the mean \pm standard deviation (n=3), p<0.05.

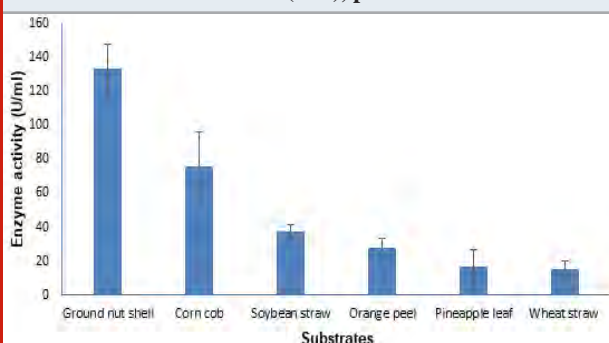
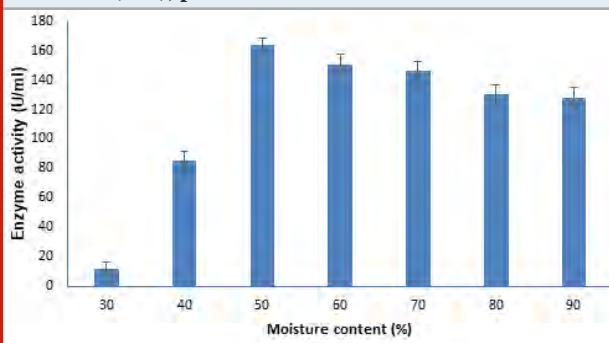


Figure 5: Influence of various moisture content on CDH production. The data are represented as the mean \pm standard deviation (n=3), p<0.05.



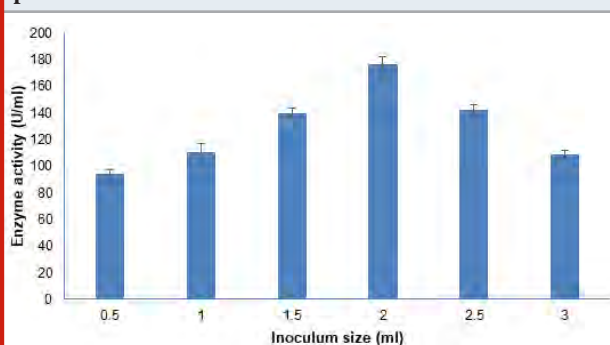
Statistical Analysis: Statistical optimization of CDH production using RSM: By using the OFAT method, the impact of different process parameters on CDH production was investigated. Four components, including moisture content, incubation temperature, particle size, and fermentation time significantly affected the enzyme production. Response surface analysis and BBD were used to establish the optimal level of significant factors to enhance CDH production by *Schizophyllum commune*.

Using a set of 29 experiments, the effects of four independent variables were examined at three levels and five central points. The results are shown in Table 2. Using Design-Expert 11.0 software, analysis of variance (ANOVA) was performed to determine the statistical significance of the model equation. The results are shown in Table. 4. A second-order polynomial equation was used to fit the obtained response:

$$Y = 2811.77 + 166.396A + 12.7667B + 138.796C + 130.629D + 0.0127083AB - 4.36167AC - 0.108AD + 0.3355BC + 0.129875BD + 5.926CD - 10.011A^2 - 0.163173B^2 - 163.731C^2 - 2.38211D^2$$

Where A represents fermentation time, B represents moisture content, C represents particle size and D represents incubation temperature. The effect of experimental variables on enzyme production was correlated using this equation. Multiple linear regressions were used to estimate the model coefficients, and those with (P<0.05) were determined to be significant. The experimental design revealed that the highest CDH activity was 217 U/ml at 50% moisture, 8th day of fermentation, 1 mm groundnut shell particle size, and 30 °C temperature. These results revealed that the predicted and experimental values did not differ significantly, indicating that the model is appropriate for maximizing CDH production. The model F-value of 6.01 indicated that the model is significant (P< 0.0009).

Figure 6: Influence of different inoculum sizes of *Schizophyllum commune* on CDH production. The data are represented as the mean \pm standard deviation (n=3), p<0.05.



The "Lack of Fit F-value" of 0.1241 indicates that the data fits the model and that the Lack of Fit is not statistically significant when compared to pure error, which is the desired characteristic. The determination coefficient was used to evaluate the model's quality of fit (R²). In this investigation, the model's R² value was determined to be 0.857; the adjusted and predicted R² values were computed as 0.714 and 0.635, respectively, with a difference of less than 0.2. This showed that this model can account for only 0.08% of the overall variations. As a result, the current R² value shows that the trial runs were accurate and consistent, and the model is reliable for CDH production.

Response surface plot: Response surface plots demonstrate the interactions of variables (Figure 10). Each response surface plot in this instance depicts the impact of two

independent variables while maintaining the levels of the other variables at zero. When the effect of fermentation time and moisture content were plotted against the enzyme activity, a link was seen; as a result, increasing moisture content with extended fermentation times encourages reaction up to the optimal level (Figure 10a). Increases in moisture content of the solid state medium up to 50% were shown to increase CDH production, which was followed by decrease in enzyme production. Similar to this, de Castro et al. (2015) employed 50% moisture to produce protease using *Aspergillus niger*. The response plot revealed that extending the fermentation period until 8th day favored maximum CDH production by *Schizophyllum commune*. Fermentation time plays a significant role in commercial production of enzymes.

Figure 7: Influence of different incubation temperature on CDH production The data are represented as the mean \pm standard deviation (n=3), $p < 0.05$.

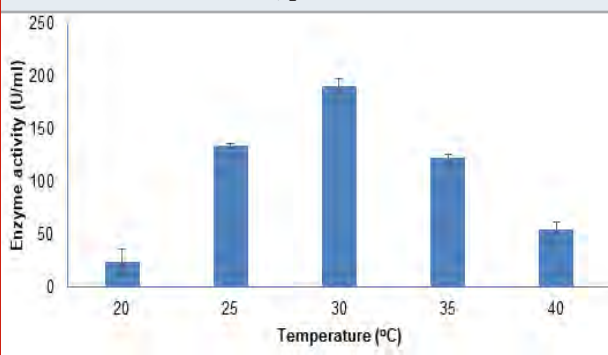
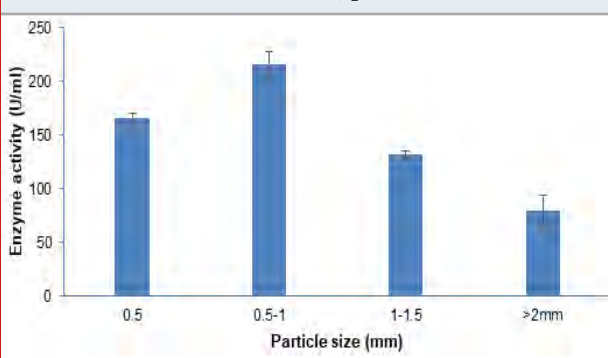


Figure 8: Influence of different particle size of groundnut shell on CDH production. The data are represented as the mean \pm standard deviation (n=3), $p < 0.05$.



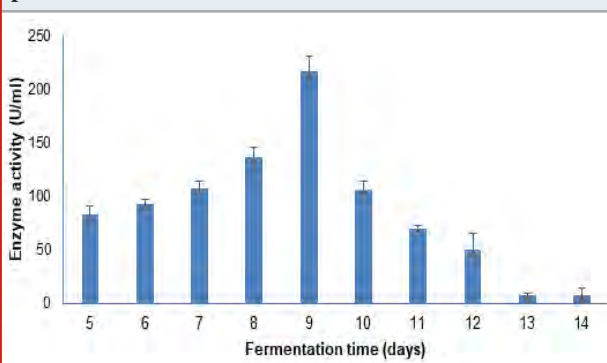
The optimal temperature was found to be 30 °C for the production of CDH based on the response surface plots, which indicated that increasing the temperature enhanced enzyme production (Figure 10c, 10e, 10f). Similar findings were observed when *Aspergillus niger* produced xylanolytic enzyme in submerged fermentation at 30 °C using RSM methods for optimization (Pellieri et al. 2022).

The response surface plots (Figure 10b, 10d, and 10f) represent the interaction between particle size of groundnut shell with fermentation time, moisture content and temperature, respectively. Here, increasing groundnut shell

particle size initially enhanced CDH synthesis; however, an optimum level was predicted at 1 mm, and after that reduction was seen. Although the particle size of ground nut shells and temperature were found to have a slightly inclined curve (Figure 10f), the graph shows that there is only minor interaction between these variables and particle size. Similarly, Prakasham et al (2006) reported 1.0 to 1.4 mm optimum particle size of green husk for protease production.

Validation of the experimental model: Fermentation was carried out under the predicted conditions to validate the experimental model. The optimum values of four variables under consideration are fermentation time 8th day, moisture content 50%, particle size 1mm and temperature 30 °C. Under optimal conditions, CDH production was predicted to be 192.75 (U/ml), however, it was actually measured at 217.88 (U/ml). There were many similarities between the actual and predicted experimental results, hence the proposed model is accurate and highly successful. The optimization of different production variables by statistical method resulted in 1.63-fold enhancement in the CDH production, as compared to results (133.3 U/ml) obtained in unoptimized conditions.

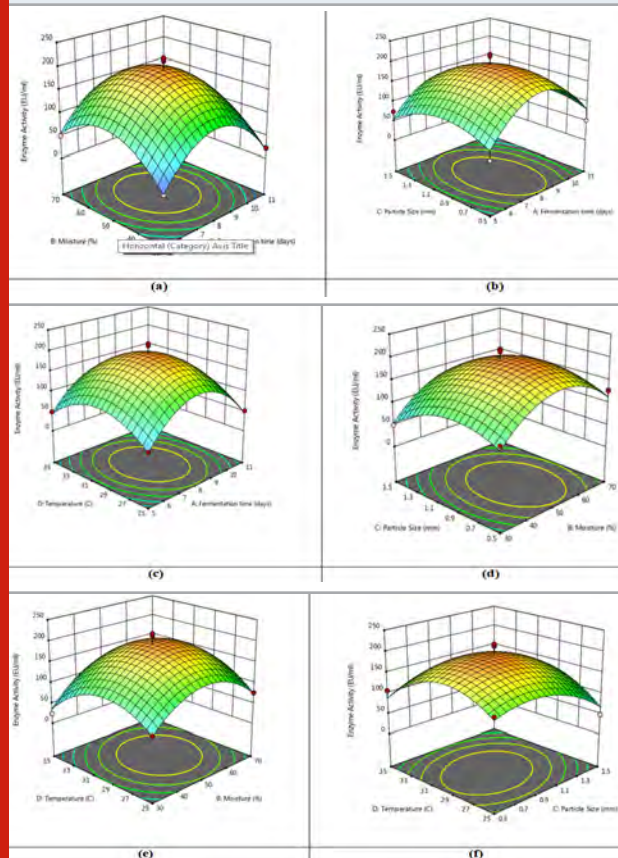
Figure 9: Influence of different incubation time on CDH production by *Schizophyllum commune* The data are represented as the mean \pm standard deviation (n=3), $p < 0.05$.



OFAT approach is difficult as it involves multiple parameters which need to be managed simultaneously, hence RSM is the preferred method for optimization (Lahiri et al. 2021). CDH isolated from *Coprinellus aureoconulatus* was used for the degradation of rice straw, where fungal hydrolases and metabolites were identified and further optimized using the RSM statistical approach (Nghi et al. 2021). Nawawi et al (2022) used Central Composite Design (CCD) model to enhance 1.34-fold xylanase and 5.96-fold pectinase production as compared to OFAT approach. Alves et al (2022) investigated optimum conditions for the multi-enzymatic recovery of cellulases produced by *Aspergillus niger* using sugarcane bagasse. The ultrasound effects were evaluated using a Doehlert design while temperature, time, and pH were analyzed using the Box–Behnken design (Alves et al. 2022). Box–Behnken Design was adopted by Mishra (2016) to optimize the fermentation conditions using *Brevibacillus brevis* and a 1.5-fold increase in protease production was achieved Gupta et al (2014) observed that

both organic and inorganic nitrogen sources affected CDH production, the optimization of these nitrogen sources was carried out using BBD. The present study is the first ever contribution to the use of groundnut shell as a solid substrate for the production of CDH using *Schizophyllum commune* and its statistical optimization.

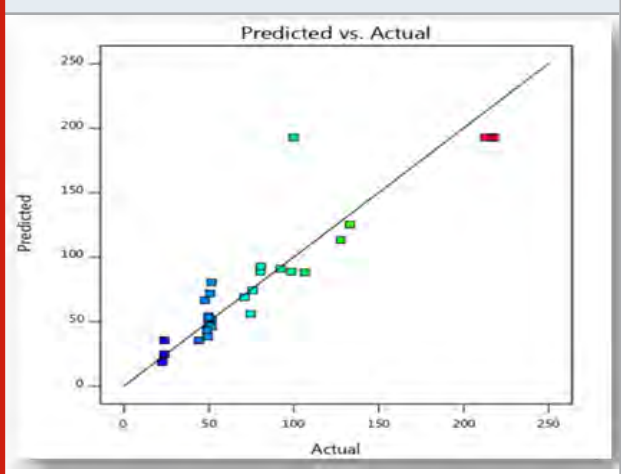
Figure 10: Response surface plots illustrating the interactions between (a) Fermentation time and moisture content (b) Fermentation time and particle size (c) Fermentation time and incubation temperature (d) Moisture content and particle size (e) Incubation temperature and moisture content (f) Incubation temperature and particle size



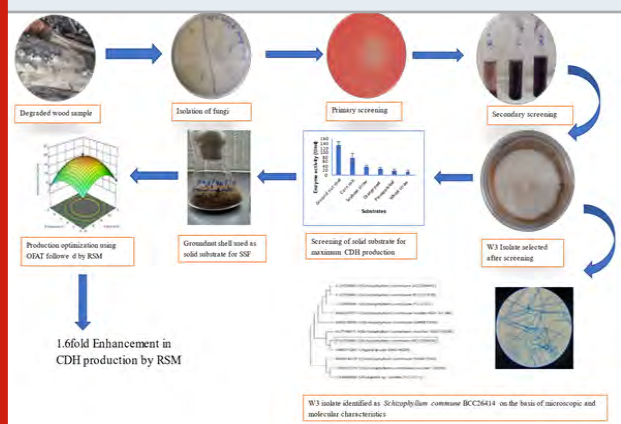
CONCLUSION

In the present study, *Schizophyllum commune* BCC26414 has been used for CDH production by solid-state fermentation (SSF). CDH production has been optimized and maximum CDH production was obtained when groundnut shell was used as a substrate at 30°C on the 9th day of incubation with initial moisture content 50%, using *Schizophyllum commune* BCC26414. This is the first report on CDH production and its statistical optimization by using groundnut shell as solid substrate. The groundnut shell is a waste from groundnut, abundantly cultivated in India. In 2020, groundnut production in Madhya Pradesh was 0.35 million tonnes. A potential strain like *Schizophyllum commune* BCC26414 can efficiently use this biowaste to produce the commercially significant CDH. Hence this

Figure 11: Graph illustrating the relationship between predicted and actual values serves as experimental validation of the CDH production model.



Graphical abstract



study can be useful in the cost-effective production of CDH for various applications.

ACKNOWLEDGMENTS

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Using Gene Expression Profile to Extract the Biomarker Genes of Cardiovascular Disease

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ABSTRACT

Cardiovascular disease (CVD) is the world's premier cause of morbidity and death. CVD is a class of heart or blood vessel diseases. CVD contains the coronary artery disease (CAD) such as unstable angina (UA) and myocardial infarction (MI) diseases. Clinicians use additional tools to support clinical evaluation and improve their ability to detect the susceptible patient at threat for CVD. Biomarkers are one such method to identify potential risk persons, rapidly and reliably diagnose disease symptoms that efficiently predict and treat disease. Discovery of MicroRNAs (*miRNAs*) representing a class of small, non-coding RNA molecules opens interesting opportunities to use the patterns of *miRNAs* as a biomarker for cardiovascular diseases. The objective of this study is to define miRNA and genes potentially associated with MI. Rothman dataset includes 52 samples of Acute Coronary Syndromes (ACS), including 18 patients with myocardial infarction (MI) and 8 patients with unstable angina (UA). Overall (number of genes selected) candidate *ncRNA* biomarkers have been defined and a *ncRNA*-based classifier has been created to predict MI risk which based on 7 *ncRNA* expression data using vector support machines SVM and decision tree classifiers. The experimental results obtained through applying these mechanisms on the Rothman dataset. The classification model's performance is evaluated using the V-fold validation and LOOCV methods. The outcome of this search can be used by the drug designer for pathway analysis and CVD treatment decisions.

KEY WORDS: CARDIOVASCULAR DISEASE, MYOCARDIAL INFARCTION, BIOMARKERS, GENE EXPRESSION, CLASSIFICATION.

INTRODUCTION

Cardiovascular disease is one of the world's leading causes of death, including coronary artery disease which resulting due to reduced blood flow into the coronary arteries caused by thrombus formation. The consequences of coronary vasospasm, which may lead to myocardial infarction (MI) as a result of myocardial tissue necrosis. Many CVD patients are not diagnosed immediately, and the prognosis is very poor, as stated in 2013, the total CVD death rate was 222.9 per 100,000 individuals (Mozaffarian et al., 2016). Several researches have been conducted in the field of genetic biomarkers for CVD, and circular RNAs have obtained significant attention due to their clinical and biological use in the diagnosis and treatment of CVD.

Over the past few decades, patients have usually been tested with an initial assessment and assessed with a risk rating or prediction algorithm that takes into account medical history, physical examination and other indicators (Čulić

et al., 2002). Additional tests, including electrocardiogram (Slater et al., 1987), coronary computed tomographic angiography (Goldstein et al., 2011) were applied to these evaluations. The existing methods, however, are inadequate for a precise diagnosis and highly sensitive, particularly in the distinction between MI and UA. Furthermore, there is a silent myocardial infarction phenomenon that is predicted to happen in about 64% of cases where patients have no chest pain or any other symptoms (Valensi et al., 2011, Gangwar 2017, Zaiou & Bakillah 2018, Guo et al. 2019 and Xu & Yang 2021).

Recent years have seen a growing interest in studying the mechanism of gene expression in which DNA instructions are translated into non-coding RNAs (*ncRNAs*), that are regarded to be essential epigenetic regulators of several physiological and pathological conditions, including cardiovascular diseases such as the acute coronary syndrome. Massive attempts have been made to use these RNA molecules as predictive biomarkers for a number of diseases including cardiovascular disease (Gangwar 2017, Zaiou, Bakillah 2018 Guo et al., 2019, Xu & Yang 2021).

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MATERIAL AND METHODS

Microarray technique was used to measure the expression of genes. The method of selection of features that led to a reduction in the volume of data used to identify biomarkers which improves the performance of the diagnostic workbook. So far, very few studies have looked at ACS subtype MI and UA, in terms of mRNA expression as a biomarker of Cardiovascular disease. In this project, I have used MI and UA samples that are sorted based on specific features. Then I filtered these samples by using

information gain algorithm to select features. The central objective of this research is to classify gene expression using classification algorithm to extract the biomarker of CVD. In this research, I have applied filter and wrapper methods to the analysis of the microarray gene expression profile. In addition, there is a previous study that used the same dataset but different classification methods to distinguish the biomarkers of cardiovascular disease. The aim of this study is to improve the accuracy of the previous study by using different classification methods, which it exceeded by the percentage of accuracy 100 % with SVM and 91% with decision tree.

Table 1. The feature selection and classification methods that used in related works

Related Works	Dataset								Feature Selection	Classification	genes	Accuracy
	GSE20601	GSE20690	GSE29111	GSE29532	GSE48060	GSE49025	GSE62					
(Kazmi, N., et al. 2016)			*		*	*	*		P-Value t-tests	-k-nearest neighbor (KNN) -(LOOCV)	79	100% 88%
(Cheng, M., et al. 2017)	*	*							p-value -Recursive feature elimination (RFE) algorithm	-(SVM)	52 87 41	84.6% 90.8% 96.9%
(Wu, K., et al. 2018)				*	*				P- value - Gene Ontology (GO) and Kyoto Encyclopedia of Genes Genomes (KEGG)	generalized linear modeling (GLM)	48 21 10	95% 70% 94%
(Lu, Y., et al. 2018)			*						-DAVID Bioinformatics Tool -(GO) -Kyoto Encyclopedia of Genes and Genomes (KEGG)	-Random forest - SVM -(LOOCV) (ROC) (AUC)	7	90.38%
(Guo, S. Z., et al. 2019)			*						- DAVID tool - fold-changes	-(SVM) -GBA pathway prediction	3	80%
									(FC) with log2 base (logFC)	method -the mean AUC across all pathway		

Literature Review: In order to present the various algorithms that have been examined. Finding the impacted and overexpressed genes for the diseases and then using association rules and gene intervals to classify gene expression. For this purpose, the researcher must know the basic knowledge for the implementation of bioinformatics strategies, including statistical methods, genetic selection techniques, associative classification algorithms and cross validation methods. Kazmi & Gaunt (2016) discussed the genomic tools and technologies that used to extract the biomarker of heart disease by analyzing the gene expression of a blood cells. The initial features were discovered by fitting a linear model for each probe collection across all arrays of patients with myocardial infarction and healthy individuals.

Three separate feature optimization algorithms were developed that specified two different sets of genes, one using MI and unstable angina, and the other using MI and normal controls. The experimental results show that the analysis of gene expression profile with the microarray study is successfully diagnoses biomarkers of some heart disease including myocardial infarction disease. It has also been shown to be effective in discriminating myocardial infarction in patients with clinical symptoms of cardiac

ischemia but without stable coronary artery disease or myocardial necrosis, (Guo et al. 2019 and Xu & Yang 2021).

The limitation in the study was that the specificity measures for some experiments could not be fully justified due to a limited number of samples. In (Cheng et al., 2017) the author presented the reasons for coronary artery disease (CAD) leading to increased mortality, angina and myocardial infarction. The dataset was processed by identifying the differential expressed genes (DEGs) and then clustering the result using the pheatmap package in R. They show the protein coding gene MAP1B and the ARG1 gene that provides instructions for the production of the enzyme arginase that appears to be indirectly regulated by CDKN2B-AS through miR-92a in the pathogenesis of CAD. This research needs to be improved through the use of empirical research to confirm these results. Wu et al., (2018) reported on the causes and treatment of myocardial infarction (MI) by analyzing miRNA that represent a signature expression pattern of miRNA that plays a vital role in MI, and also by analyzing the protein-protein interaction network, which plays a role in the understanding of the MI molecular mechanism. The results of the experiment have been validated using RT-PCR.

Table 2. Microarray datasets details used on related works.

Dataset	URL	Samples	Genes	Database
GSE20681	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20680	198	45015	NCBI Gene Expression Omnibus (GEO)
GSE20680	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE20680	195	45015	
Rothman Dataset: GSE29111	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29111	52	54675	
Gregg Dataset: GSE49925	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49925	338	14111	
Beata Dataset: GSE62646	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62646	98	33297	
Nelson Dataset: GSE48060	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48060	52	54675	
GSE29532	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29532	55	22011	

The relationship of the non-coding miRNA to the expression of target genes. This study contributes to the understanding of the MI mechanism by supporting clinical diagnostic and the development of appropriate treatment for MI. The drawbacks of this study in the lack of research samples and the need for confirmation of this analysis by experts from other disciplines such as cytology. Lu et al., (2018) reported that there's little understanding about the patterns and function of lncRNA in the interduce of acute coronary syndromes (ACSs). Dysregulated lncRNA expression has been involved in cardiovascular disease. The lncRNA expression profiles were examined in the two distinct clinical entities of ACS, unstable angina (UA) and myocardial infarction (MI). In addition, functional analysis mentioned that these candidate lncRNA biomarkers could be implicated in known MI-associated pathways and

biological processes. This research was the first in ACS growth to recognize altered patterns of lncRNA expression. this paper presents a novel functional lncRNAs may be used as therapeutic targets biomarkers and candidate diagnostic.

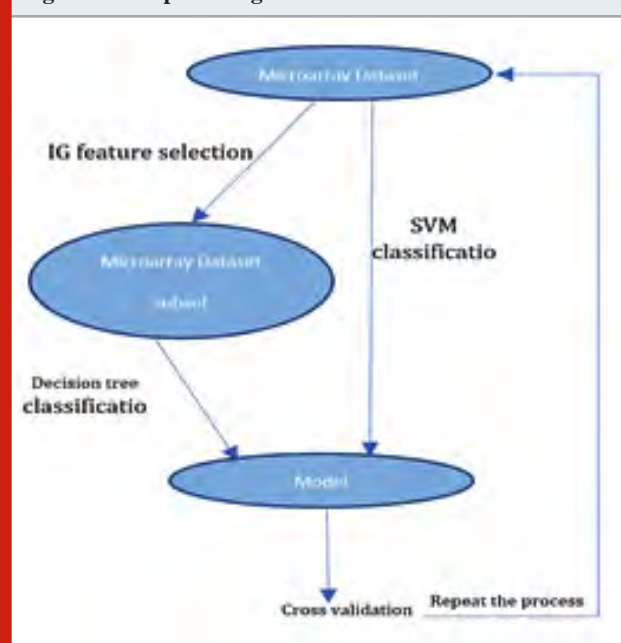
This study can be improved by conducting more experimental studies to determine the molecular mechanism of these novel lncRNAs in the progression of ACS. In (Guo et al., 2019) this study, gene data for patients with UA or MI have been used to define optimal pathways that provide extensive information for the progression of MI/ UA. The paper mentions the limitations of the existing method in disease progression information obtained from the identification of differentially expressed genes (DEGs). The paper's contribution was made by using the LIMMA Detection

Package (DEGs) between UA and MI, in addition to the differential co-expression network (DCN) and the sub-DCN for the DEGs. (DCN) proved to be a new holistic approach to microarray analysis. The importance of identifying the optimal pathways to demonstrate the progress of ACS development. This work needs to discover the underlying mechanisms for the progression of ACS using animal models.

Proposed Method: Bioinformatics is the process of collection, classification, storage, and analysis of biochemical and biological information using computers especially as applied to molecular genetics and genomics. In this section, we will define the methods that we applied on dataset which are a feature selection method for biomarker mining and classification methods which is the concept of statistical models for the diagnosis of diseases. These methods calculate the mutual information between the candidate and the class label and the average of the mutual information between the candidate and the attributes of the selected subset.

In this research paper, we have used Information Gain (IG) feature selection method and with Decision tree classification method for selecting important genes to reduce the number of genes that help to increase the speed and accuracy of prediction systems. Then we compared the resulting accuracy with SVM classification methods using LOOCV cross validation. Below we will illustrate brief description about each method. Figure 1, shows clear view about our proposed method.

Figure 1: Proposed Algorithm



Feature Selection Method: For feature selection methods we used both the filter model and the wrapper model. Information gain filter feature selection method and wrapper decision tree selection method. Filter methods use heuristics based on data characteristics to select predictive subsets of features. The wrapper model uses a mining

algorithm's predictive accuracy to evaluate a selected subset's goodness.

a) Information gain (IG): Information gain is applied as a filter in the selection of genes. It is measured by calculating the IG value by using a system entropy. Then the genes are ranked depend on its IG value. The information gain calculates the difference between the entropy before splitting and the average entropy after splitting the samples based on the given attribute values. Entropy is the proportion of the instances to its classes that calculate as the following equation: $IG = \text{information before splitting (parent)} - \text{information after splitting (children)}$.

Classification Methods: In this research we applied two classification methods. The first one is support vector machine (SVM) which is directly applied to microarray dataset. The second one is decision tree (DT) algorithm which is applied as wrapper method combined with information gain (IG) method.

a) Support Vector Machine (SVM): Support vector machine (SVM) is a category of associated supervised learning methods used in regression and classification. The simplest method (SVM) is a linear classification, which is intended to draw a line that separate data into two-dimensional.

b) Decision Tree (DT): The decision tree is hierarchically represented with the root at the top and split into branches / edges until the end of the branch that no longer splits is the decision / leaf. The structure of decision tree where the branches represent conjunctions and the leaves represent classifications of features. Previous assumptions about the nature of the data are not required, therefore this method can classify the numerical and categorical data. The output attribute must be unique in the decision tree algorithm. In the situation of changing training data, different attributes selection produced with selecting point in the tree.

Cross validation: It is necessary to test data samples independently of the learning dataset used to build a classifier, in order to perform a classification error measurement. V-fold cross-validation technique performs independent tests without requiring a separate test dataset that lead to increases the difficulty and price of the test and use this technique without reducing the data was using to construct the tree. The learning database is split into 10 folds of cross-classification rows groups.

a) Leave-one-out cross-validation (LOOCV): Includes the sample divided into validation data for a single observation and training data for the remaining observations. This contributed to the quality of the classifier based on lncRNA, including its precision, sensitivity and specificity.

RESULTS AND DISCUSSION

In this section we will discuss the gene expression dataset used in our research. Then, we will present the experimental result of our proposed method and comparison with more related work. In this research, (GEO; GSE29111) Rothman

dataset includes 52 samples of Acute Coronary Syndromes (ACS), including 18 patients with myocardial infarction (MI) and 8 patients with unstable angina (UA). The number of (MI) samples is 26 and the number of (UA) samples is 16. The total number of the attributes are 54675.

The Rothman dataset contains samples of whole blood mRNA expression obtained from 26 acute coronary syndrome (ACS) patients, collected in two periods of time at 7 days and at 30 days post-ACS. MI patients were compared with those with UA (not healthy controls), thereby concentrating on variations in mRNA expression due to acute clinical conditions rather than underlying atherosclerosis and its treatment. Datasets were processed on the Affymetrix Human Genome U133 plus 2.0 platform. The robust multi-array averaging (RMA) method of the "affy" package in R was used to normalize the dataset.

Experimental Discussion: we used the open-source Anaconda Distribution to perform Python to apply feature selection and classification methods. Online converter was used to convert dataset format from text original format file into four excel files due to its large size, which was read as a single file in Python. The subjects of the Rothman dataset

divided into testing and training sets to define the final classifier among all optimized lists. It proposed to classify MI patients from patients with unstable angina.

SVM classification methods was first applied to the data set without features selection method, we got only 6 genes and the classification accuracy was 91%. Revealed the effects of SVM classifier by applied 10-fold cross validation which result the overall accuracy of the model with append each score to a list and get the mean value -0.108. Then applied LOOCV to get more validation.

After that we compiled the information gain feature selection (IG) with decision tree method (DT). The IG method with decision tree algorithms were applied to the dataset using scikit learn package. Decision tree algorithm go throw selection best attribute using attribute selection measure (ASM) which (IG) measure. Then, spilt the dataset into smaller dataset with 7 genes and recursively for each child until one condition match the lowest accuracy. The classification. accuracy of information gain (IG) with decision tree (DT) algorithm was 100%. The result of this classification is overcoming the previous study as shown in the Table 3.

Table 3. Comparison between our proposed method and the most related work in literature based on classification accuracy and number of selected genes

Methods	Gene Selection Method	Classification Method	Dataset	Number of Genes	Classification Accuracy
Kazmi, et.al (2016) [8]	- P-Value - t-tests	- K-nearest neighbor (KNN) (k = 3). Using (LOOCV)	-Rothman	7 9	100% 88%.
Our Proposed Methods	Information gain (IG)	- Decision Tree (DT) - SVM Using (LOOCV)	-Rothman	6 7	91% 100%

CONCLUSION

To conclude, bioinformatics analyzes were carried out on GSE29111's microarray data set to explore CAD's genetic mechanisms. This data set was previously used with different classification and selection methods than our research study, which improved the accuracy of classification by applying the decision tree, including the IG selection method, in addition to the SVM classification method. However, in future, further classification methods need to be applied in order to improve these findings.

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Molecular Identification of Medicinal Plant, *Polygonum posumbu* from Manipur, India

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ABSTRACT

Polygonum is an important genus of Polygonaceae and *Polygonum posumbu* is underutilized traditional medicinal plant whose leaves are extensively used as an antipyretic and dyspepsia agent in Manipur, India as well as a spice in India, Japan, Nepal, China, South Korea, Philippines and Thailand. *P. posumbu* is commonly known as Phak-pai in Manipur. DNA barcode based molecular characterization technology for species identification has been recognized as a reliable tool for plants but the selection of suitable universal marker is still under discussion. The main aim of the study is to identify the *Polygonum posumbu* sample from Manipur, India using DNA barcode method. In the present study, *Polygonum posumbu* samples were collected from a local farmer in Awang khunou, Imphal West, Manipur, India which was taxonomically identified. To assess the molecular species identification, Internal Transcribed spacer (ITS) marker system was employed and the primer's pair used were covered the internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2 complete sequence. Phylogenetic tree was constructed for the studied sample with the sequences of the 9 species retrieved from the GenBank in order to show the relationships among the samples. The result shows that the ITS marker-based DNA identification method successfully characterized the *Polygonum posumbu* species. The information of ITS marker-based identification of *Polygonum posumbu* from Manipur, India, will enhance our knowledge in better understanding the medicinal properties of this plant.

KEY WORDS: DNA BARCODE, IDENTIFICATION, ITS MARKER, PHYLOGENETIC TREE AND POLYGONUM POSUMBU.

INTRODUCTION

According to reports, North-East India has used more than 2000 kinds of ethnobotanical plants to make a variety of remedies (Schori and Showalter 2011). *Polygonum posumbu* Buch.-Ham. ex D. Don, a small herb, which is distributed in the North-Eastern states of India. Manipur is one of the North-Eastern states and here *P. posumbu* is used as an ethnomedicinal plant in various ways. Traditionally, tender shoots of *Polygonum posumbu* is consumed as a spice and leaves are used as mouth-freshener (Asha et al. 2011). As a folk medicine, it is used for curing dyspepsia and fever (Singh et al. 2003; Yumnam et al. 2012). The juice of the tender shoots and fresh leaves are used to reduce heartbeat (Leishangthem and Dinendra 2014 Reshmi Singh et al 2016, Mosa et al 2019, Cahyaningsih et al 2022).

This plant also possesses antibacterial activity against *Clostridium sporogens*, *Neisseria gonorrhoea* and *Bacillus*

cereus (Ishwori et al. 2014). A great source of unidentified chemical compounds with therapeutic benefits is medicinal plants (Rao 2004). There is an urgent need for precise taxonomy inventorization in relation to species-level genetic characterization of the plant from this region, as the majority of the people in this place used the traditional knowledge of herbal medicine. Despite the medicinal value of *Polygonum posumbu* in traditional medicinal system, this plant does not have any other information including pharmaceutical, genetics and genomics information.

The main concerns in ethnobotany focused on the significance of accurate species identification as well as the interpretation of indigenous and conventional knowledge of restorative plant usage and their transfer for the advancement of bioprospect in human health care. Currently, the re-evaluation of traditional medicine is being done all over the world via comprehensive research on various plant species and their therapeutic properties. Consumer demand is currently encouraging the expansion of dietary supplements and innovative herbal plant-based treatments. In the healthcare sector of the 21st century, use of herbal

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plant based dietary supplements or medication is rapidly growing (Siew et al. 2014, Cahyaningsih et al 2022).

Due to misidentification, there is the probability of substitution within certain plants which may give different affects rather than the expected certain properties from that of the original plant. Therefore, proper identification of herbal medicinal plants in relation to their naturalness and lack of adulteration and safe application has become increasingly important (Pang and Chen 2014). An ideal strategy for the preservation of plant genetic resources and genetic advancement is the characterization of plants using morphological and molecular markers (Rout and Mohapara 2008). Both molecular and morphological characteristics are important for the identification of underutilized medicinal plants and the combined data would provide more comprehensive information for those plants.

The appraisal and exploitation of genetic resources can also benefit from the use of morphological and molecular data. Plant genetic diversity has been described and evaluated using morphological characteristics. However, morphological characteristics, particularly quantitative traits are not stable as environmental conditions from one region to other region may have impacts on the morphology of the plant. The majority of the current taxonomy plant group identification is morphological in nature. Nevertheless, this identification technology has several limitations when attempting to distinguish between plants at different developmental stages. Since molecular markers are not affected by environmental factors, they are a useful complement (Solmaz et al. 2010, Cahyaningsih et al 2022).

DNA barcoding has become a highly effective method for identifying species, as seen by the widespread use of this method in bioresource monitoring and documentation (Kress et al. 2005; Hollingsworth et al. 2011; Stoeckle et al. 2011 and Liu et al. 2012, Cahyaningsih et al 2022). The technique's application stresses some key areas, such as documenting the significant and fragile ethnomedicinal plant bioresources, with which the field of "Ethnobotany Genomics" has just been created (Newmaster et al. 2010). The purpose of DNA barcoding is clearly to identify an unknown sample using pre-existing classification but it will not detect relationship between samples. The basic goal of DNA barcoding is to identify a universal DNA sequence that balances conserved sequences with enough variability to distinguish across different organisms. The basic materials used to create herbal goods can be identified using barcoding technology.

The "Consortium for the barcode of life plant working group (CBOL)" has accessed numerous chloroplast genomic areas (ITS, 5S rRNA, rbcL, rpl36-rps8, matK and trnH-psbA) in plant systems (CBOL 2009). Among the chloroplast genomic areas used for plant identification, internal transcribed spacer (ITS) or its segments (ITS1, ITS2) is one of the most frequently used regions in plant molecular systematic at generic and species levels. This is due to the fact that, ITS has the potential to provide a clear differentiation of relationships between and within species

(Hillis and Dixon 1991; Yuan et al. 2015; Buchheim et al. 2011; Alvarez and Wendel 2003; Staggermeier et al. 2015; Keller et al. 2010 and Baldwin et al. 1995).

It was used as a seed plant barcode (Song et al. 2012; Hollingsworth 2011 and Li et al. 2011). Studies showed that, species identification using barcodes from both the uniparentally inherited plastid genome and biparentally inherited nuclear genome is more accurate or even required and the nuclear genome's ITS region is now the most promising candidate (Fazekas et al. 2009; Chase and Fay 2009 and Roy et al. 2010).

An example for the use of DNA barcoding genes for identification of species is the illustration of the discrimination of *Schisandra chinensis* at the species and population levels with the use of ITS2 (Li et al. 2013). Additionally, *S. Chinensis* from *S. sphenanthera* could be distinguished by their ITS2 region with great clarity. In a study to identify Astragalus 319 species were examined across four coding (trnH-psbA, rpoC1, rbcL and matK) and two non-coding (ITS and ITS2) areas; ITS2 and ITS barcodes were more effective at differentiating the species (Gao et al. 2009). The situation where *Peucedanum praeruptorum* has been distinguished by DNA barcoding. In order to identify it from typical knockoffs and adulterants, Zhou et al. 2014 employed ITS and nrDNA barcodes. Despite the fact that, the *Polygonum posumbu* has high traditional economic importance, owing to their medicinal and nutritional value, till date, there is no information concerning chemical constituents as well as genetic information, and pharmacologic activity of the plant. Therefore, the main purpose of our study was to identify *Polygonum posumbu* plant commonly found in Manipur, India. The data will be helpful in further exploration of *Polygonum posumbu* and also in functional genomics for improving the medicinal uses of *P. Posumbu*.

MATERIAL AND METHODS

Sample collection: For the study, healthy *Polygonum posumbu* plants were collected from a local farmer in Awang khunou, Imphal West, Manipur, India (N 24°47.177' E 093°50.981') in November 2017. Leaf tissues of plant samples were cleaned, and then stored in a sterile polyethylene bag at -80°C until further analysis. For morphological identification herbarium was prepared using 4% formalin. The plant sample was identified based on morphological and taxonomical characteristics by Dr. B. Thongam, Taxonomist, IBSD, Manipur, India. A voucher specimen was deposited in the herbarium of the Institute of Bioresources and Sustainable Development (IBSD), Manipur, India. The plant sample was also identified by the Botanical Survey of India, Eastern Regional Centre (BSI ERC), Shillong.

DNA isolation: Fresh plant leaves were washed with sterile distilled water and scraped. Approximately 100mg of leaves were sliced into thin pieces and ground to a fine paste using liquid N₂. Total genomic DNA was extracted by using DNeasy Plant Mini Kit (Qiagen). The isolated DNA was purified and quantified in gel electrophoresis using gel red

stained band intensity and concentration and purity of DNA were checked in Bio Spec Nano (Shimadzu).

DNA amplification: The polymerase chain reaction (PCR) was performed using ITS-An5, 5'-CCTTATCATTAGAGGAAGGAG - 3', ITS-An4, 5'-CCGCTTATTGATATGCTTAAA - 3' primer pair (Table 1). The PCR reaction of 25µl mixture consisted of 10X PCR reaction buffer (2.5 µl), 25mM MgCl₂ (1.5 µl), 10 mM dNTPs (0.5 µl), 5 unit of Taq polymerase (0.25 µl), 10µM of each primer (1 µl), 50ng genomic DNA (1 µl) and 20.25µl Milli-Q water (17.25 µl). PCR thermal conditions (on a C1000 Touch Thermal Cycler; Bio-rad) were 95°C for 3 min, 32 X [95°C for 30s, 52°C for 1 min, 72°C for 1min] and final extension at 72°C for 5min. The PCR product was checked by 1.5% agarose gel electrophoresis.

Purification of PCR products and DNA sequencing: Qiagen DNA Mini Kit (Germany) was used to purify the PCR product to ensure that the final products were free of contaminants. Purified PCR products were sent for Sanger sequencing at Europhins Genomics India Pvt., Ltd., Bangalore.

Sequence analysis: Utilizing BLASTn analysis, the ITS amplified sequence was BLAST in GenBank. The forward and reverse sequences were then aligned to the target sequences and raw sequences were edited manually. The aligned sequences were corrected manually and checked the nucleotide composition. For a sequence length of ~724bp, the terminals 3' and 5' were cut to provide consensus sequence for the taxa. The modified sequence for the studied species was submitted to NCBI databases (<http://www.submit.ncbi.nlm.nih.gov>). Also, 9 sequences for the same or related species as the examined material were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/genbank>).

Phylogenetic analysis: The ITS region of 9 taxa from the Polygonaceae family was retrieved from GenBank for the phylogenetic tree construction in which 8 taxa are from same genus *Persicaria* and the 9th one is from the genus *Antigonon* (name of the species is *Antigonon leptopus*). The phylogenetic tree was constructed for the studied species and UPGMA analysis was done in Mega 11 software to examine the relationship between the studied species and 9 taxa retrieved from GenBank and bootstrap value of 1000 replicates was calculated.

RESULTS AND DISCUSSION

In this study, fresh plant sample of *Polygonum posumbu* collected from Manipur, India was characterized based on morphological traits. The voucher specimen number (IBSD/M-251) (fig. 1) and (No: BSI/ERC/Tech/2021/111) were assigned respectively from the Institute of Bioresources and Sustainable Development (IBSD), Manipur, India and Botanical Survey of India (BSI), Eastern Regional Centre (ERC), Shillong respectively.

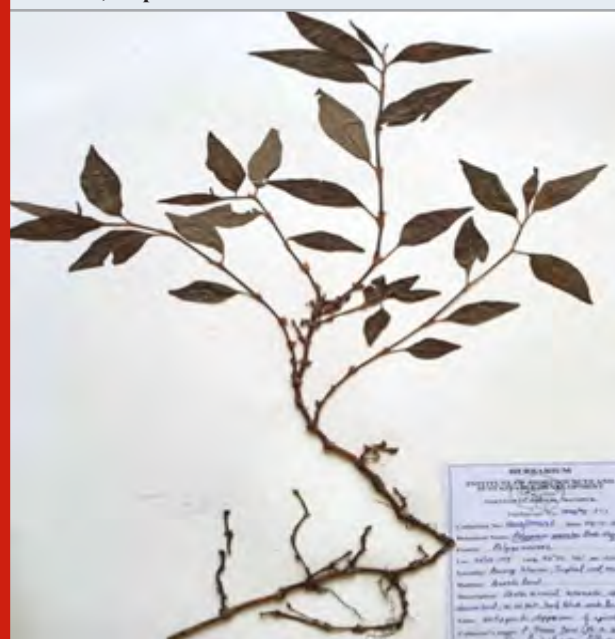
Species identification: *Polygonum posumbu* has been identified using ITS primer pair ITS-An5 and ITS-An4. In this investigation, the amplification of the primers was

satisfactory and reproducible. Due to length variation in the ITS forward and reverse sequences, only 724 aligned nucleotide positions were used for sequence analysis. According to BLASTn analysis, the sample was accurately identified upto the species level proving that the preliminary identification of plant based on morphological and taxonomical characters matched with the scientific name received from GenBank. BLASTn analysis result for the sample investigated shows a maximum identity of 98.90% and query coverage of 100% which is showing that ITS marker-based identification of *Polygonum posumbu* obtained from Manipur is accurate (Table 2). The accession number of the sequence submitted to the NCBI databases is OP602225.

Table 1. Sequence of primers used for molecular identification

S. No.	Primer code	primer sequence	Nucleotide length (bp)
1.	ITS-An5	CCTTATCATTAG AGGAAGGAG	22
2.	ITS-An4	CCGCTTATTGATA TGCTTAAA	21

Figure 1: Taxonomic identification of *Polygonum posumbu* in IBSD, Imphal



Phylogenetic analysis: UPGMA tree construction methods were used to construct dendrogram for the sequence of the studied sample and 8 sequences from same taxa and 1 sequence from different taxa (Table 3). The dendrogram derived from UPGMA analysis of the Maximum composite likelihood of all the 9 sequences with the sequence of the studied sample showed that the studied sample collected from Manipur, India (OP602225, *Persicaria posumbu*) is clustering with the sequences of the same taxa forming their

own grouping and other 8 sequences are clustered away from the studied sample (Fig. 2).

In the present study, morphological identification was carried out first in order to identify the *Polygonum posumbu* collected from Manipur, India. However, morphological variability is frequently constrained, characteristics may not express at all the phases of plant development

and appearance may be influenced by external factors. The therapeutic potential of medicinal plants will be compromised by incorrect plant identification, affecting the human health. There have been numerous reports of toxic cases, most of which were caused by species misidentification (Viljoen 2013). The issue of an increase in herbal treatments that have been tampered with or replaced with other plant materials has highlighted the necessity of quality monitoring (Raterta et al. 2014).

Table 2. BLAST analysis result of ITS sequence of *Polygonum posumbu* (studied sample)

Sample sent to NCBI	maximum identity	query coverage	sequence length	Accession No.
Persicaria posumbu Voucher BSI/ERC/Tech/2021/111	98.90%	100%	724 bp	OP602225

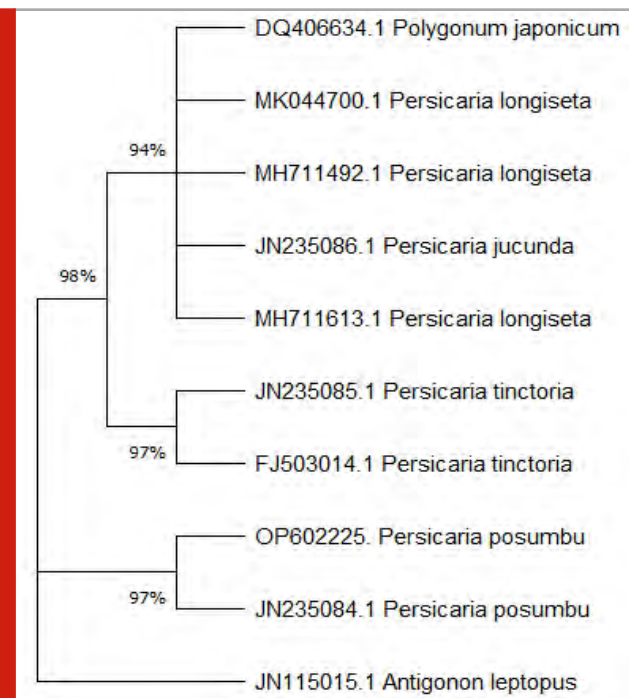
Table 3. ITS sequences of Polygonaceae family retrieved from NCBI

Species	Genus	Accession No. of ITS
Persicaria posumbu	Persicaria	OP602225 (studied sample)
<i>Persicaria posumbu</i>	Persicaria	JN235084.1
<i>Polygonum japonicum</i>	Polygonum	DQ406634
<i>Persicaria jucunda</i>	Persicaria	JN235086
<i>Persicaria longiseta</i>	Persicaria	MH711613
<i>Persicaria longiseta</i>	Persicaria	MH711492
<i>Persicaria longiseta</i>	Persicaria	MK044700
<i>Persicaria tinctoria</i>	Persicaria	JN235085.1
<i>Persicaria tinctoria</i>	Persicaria	FJ503014.1
<i>Antigonon leptopus</i>	Antigonon	JN115015.1

With regard to DNA level identification, molecular technologies provide much more useful informations. Nowadays, several PCR based approaches could be used to distinguish between species of the same genus. Today, genetic marker technology has been used by many researchers for studying genetic resources as the conventional methods have their limitations. A perfect DNA barcode should have enough conserved segments to design a universal primer, high variability to be used for species discrimination and the ability to differentiate among the closely related species. This is possible if a species has a very large genetic distance from the other intraspecific members of the group (Hebert et al. 2004; Mankga et al. 2013, Cahyaningsih et al 2022).

DNA barcode research has been extensively used for phylogenetic tree-based methods to assign species to their appropriate taxa. The most popular phylogenetic tree is NJ, and the main criteria for evaluation were morphological distance, evolutionary history and species documentation

(Liu et al. 2014). Today researchers working in the field of plant sciences have access to a variety of DNA markers.



However, the difficulty of developing a universal barcode for the identification of all plant species has been contested by several researchers, and it is because of morphological and geographical variations as well as reticulate evolution (Mosa et al. 2019). According to a review on the role of DNA barcoding as a powerful tool for plant biodiversity analysis, the ITS and rbcL genes have been acknowledged as fundamental barcode markers (Mosa et al. 2019). This further supports our assessment of 'ITS' dependability as a DNA barcode marker. However, there is no standardised barcode for plant identification, therefore researchers are using different sets of marker genes to increase the accuracy of species identification, (Cahyaningsih et al 2022).

CONCLUSION

The *Polygonum posumbu* collected from Awang- khunou, Manipur, India was successfully identified based on DNA characterization at ITS gene. The current study suggests that ITS is the reliable marker for identification of *Polygonum posumbu* from Manipur, India using the BLAST analysis genetic distance approach as deduced by phylogenetic tree. Our study for the identification of *Polygonum posumbu* plant from this region will help in further research on the important properties of this particular plant.

Ethics approval and consent to participate: Not applicable

Conflict of interests: Authors declare no conflict of interests to disclose.

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Potential Application of the Objective Regression Regressive Methodology

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ABSTRACT

The possibility of having a methodology that allows the modeling and prediction, in the short, medium and long term, of biological, social and natural disaster processes and/or phenomena is something great. The objective of the research consisted in demonstrating the potentialities and real capacity of application of the methodology of the Regressive Objective Regression (ROR) in the different fields and branches of scientific research. In the ORR methodology, in a first step, dichotomous variables DS, DI and NoC are created. Then, the module corresponding to the Regression analysis of the SPSS statistical package (ENTER method) is executed, where the predicted variable and the ERROR are obtained; subsequently, the autocorrelograms of the ERROR variable are obtained, paying attention to the maximum of the significant partial autocorrelations, and the new variables are calculated according to the significant Lag of the PACF. Finally, these regressed variables are included in the new regression in a process of successive approximations until a white noise is obtained. Wide possibilities of modeling and forecasting in the short, medium and long term, which go beyond the modeling of infectious entities of parasitic and viral etiology, Acute Respiratory Infections, Acute Bronchial Asthma crises, forecasting of extreme meteorological disturbances, prediction of latitude and longitude of earthquakes, modeling of climatic variables, and even the own electric consumption of a municipality, province and nation. The ROR methodology has demonstrated potential and real capabilities of application in dissimilar fields and branches of science, so it is a novel contribution to the science of modeling and forecasting of variables to know the future, as well as the impact that different variables contribute to an event or phenomenon, and being universal, it can be applied anywhere in the universe.

KEY WORDS: AUTOCORRELATIONS; AUTOCORRELOGRAMS; ROR METHODOLOGY; POTENTIALITIES; DICHOTOMOUS VARIABLES.

INTRODUCTION

The possibility of having a methodology that allows the modeling and prediction, in the short, medium and long term, of biological, social and natural disaster processes and/or phenomena is something great for modern science (Gore, 2007; Chandra et al., 2008; Fimia et al., 2017a). Throughout history, mankind has suffered the scourge of potentially fatal viral and parasitic diseases, among which Yellow Fever, Dengue, Zika, Chikungunya and Malaria stand out; most of them often involve a mosquito (Diptera: Culicidae) as a common factor (Chandra et al., 2008; Troyo et al., 2008, Dagogo et al., 2020, Ban, 2021).

These diseases are widespread in the tropics, with local variations in risk depending largely on rainfall, temperature

and rapid unplanned urbanization, among others (Brenda et al., 2000, WHO, 2019). In addition to these problems, global warming and the intensification of extreme weather disturbances have brought about changes in the behavior of diseases and their transmission, with the establishment of vector species in places never recorded before (WHO, 1998; Xie et al., 2006; Gore, 2007). Efforts to control vector-borne diseases have been hampered in part by the development of drug-resistant etiologic agents, insecticide-resistant mosquitoes, environmental contamination, the residual effect of chemical substances, the high prices of insecticides on the market, operational failures, abandonment of vector control programs, among other causes (Das and Amalraj, 1997, Dagogo et al., 2020, Ban, 2021).

Despite the efforts and resources that have been put into the control of disease-transmitting mosquitoes, they are still not being controlled; on the contrary, the appearance of resistance and the development of defense mechanisms

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against the increasing use of insecticides used for their control are becoming more and more evident (Ayala et al., 2008; WHO, 2014; Fajardo et al., 2016; Real, 2017). The increase in diseases transmitted by vector-borne organisms increasingly commits the scientific community to prioritize the search for much more efficient, economic, feasible and sustainable control alternatives over time, where the use of mathematical modeling, which has been applied in different fields of study, stands out in recent decades.

There are about 77 known equations referred to sigmoidal growth models, which are used in epidemics, bioassays, agriculture, engineering fields, tree diameter, forest height distribution, etc. (Dagogo et al., 2020), and where growth models accumulate and accumulate in a variety of ways, and where cumulative growth models over time have played an important role, as well as many researchers who have contributed to the knowledge of models developed in a relevant way, with emphasis on some non-linear models, among the most common are (Gompertz, Weibull, negative exponential, Richard's model (logistic, mono-molecular), Brody, Mitcherlich, von Betalanffy, S-Shaped, among others) (Ivorra and Ramos, 2020; Zhao et al., 2020; Min, 2021).

diameter, forest height distribution, etc. (Dagogo et al., 2020), and where growth models accumulate and accumulate in a variety of ways, and where cumulative growth models over time have played an important role, as well as many researchers who have contributed to the knowledge of models developed in a relevant way, with emphasis on some non-linear models, among the most common are (Gompertz, Weibull, negative exponential, Richard's model (logistic, mono-molecular), Brody, Mitcherlich, von Betalanffy, S-Shaped, among others) (Ivorra and Ramos, 2020; Zhao et al., 2020; Min, 2021). There is the possibility of making high quality, precise and accurate forecasts using several methodologies, among which the methodology of Regressive Objective Regression (ROR) stands out (Osés and Grau, 2011), which due to its simplicity and accuracy can open an important window to know the future of climatic variables or daily data, years in advance and even many more (Osés et al., 2014a; Osés et al., 2014b; Osés et al., 2017a; Osés et al., 2017b; Osés et al., 2018).

This cycle can be extended to the 11 years of the solar cycle, or to higher cycles that are known in nature; the population dynamics of mollusks and insects, such as culicidae and their interactions with certain environmental variables, can also be modeled in order to establish prophylactic and timely control measures in epidemiological surveillance programs. Consequently, there is a growing need to develop and implement other strategies and alternatives for the control of infectious entities and their vectors, which can complement existing methods in a more effective and efficient way (Osés et al., 2018; Fimia et al., 2017a; Fimia et al., 2017b; Fimia et al., 2020).

Fields of application of the ROR methodology

The ROR methodology as a function of the control of culicidae larval populations: In the specific case of

culicidae, it was possible to apply the ROR methodology to larval densities, both general (DLG) and specific (DLE) (mosquitoes of the *Anopheles* genus), as well as for the *Aedes aegypti* mosquito species, involved in the transmission of the arboviral entities Dengue, Yellow Fever, Chikungunya and Zika; it was also possible to apply this methodology to the population dynamics of culicidae in the provinces of Sancti Spiritus and Villa Clara (Ivorra and Ramos, 2020; Zhao et al., 2020; Min, 2021). On the other hand, this methodology made possible the modeling of some climatic variables (Temperature, Precipitation, Atmospheric Pressure, Relative Humidity, and Wind Speed, among others) with the mosquito population densities, and made possible the realization of mathematical modeling in the short, medium and long term. The analysis and processing of all these databases has allowed both researchers to publish more than 18 scientific articles related to this topic, from 2012 to the present, in prestigious journals with high impact factors, indexed in first level/group 1 databases and even in the Web of Science (WoS).

ROR methodology and its impact on fluvial and terrestrial malacofauna with veterinary medical interest:

For the mollusks group, research was specifically focused on the fluvial and terrestrial malacofauna of Sancti Spiritus and Villa Clara provinces, with emphasis on the following aspects: modeling and prediction of the infectious entities fasciolosis and angiostrongilosis by means of the ROR methodology in Villa Clara province; influence of some climatic variables on the fluvial and terrestrial malacofauna with zoonotic importance in Villa Clara and Sancti Spiritus. Studies on distribution and abundance of fluvial and terrestrial gastropods with meteorological variables by means of mathematical modeling, as well as the application of this methodology to the population dynamics of the fluvial and terrestrial malacofauna of Villa Clara and Sancti Spiritus.

The analysis and processing of the databases on fluvial and terrestrial malacofauna has allowed both researchers to publish six scientific articles related to this topic, in prestigious magazines with high impact factors, indexed in first level/group 1 databases and even in the Web of Science (WoS), as well as two degree theses, in the specialties of Biology and Veterinary Medicine, of the Central University "Marta Abreu" of Las Villas.

ROR methodology and its application in transmissible infectious entities:

Mainly in the application to a group of transmissible infectious entities, both of viral and parasitic etiology, such as HIV, Leptospirosis, Cholera, Dengue, Chikungunya, Yellow Fever and Zika, and the case of Malaria, as the main parasitic entity. The analysis and processing of the databases on transmissible infectious entities, allowed both researchers to publish eight scientific articles related to this topic, in prestigious and impact journals, indexed in group 1 and 2 databases, and even some of them in the Web of Science (WoS), as well as two master's theses, one defended at the University of Medical Sciences (UCM-VC), and the other at the Central University "Marta Abreu" of Las Villas.

The ROR Methodology Applied to Acute Respiratory Infections and Bronchial Asthma Crises: The possibility of mathematical modeling one year in advance for Acute Respiratory Infections (ARI) and Acute Bronchial Asthma Crises (CAAB), as well as the impact of temperature on the appearance of respiratory infections in birds in a tropical country. All of which gave us the possibility of publishing four scientific articles related to this topic, in prestigious journals of recognized prestige and impact, indexed in group 1 and 2 databases, and even some of them in the Web of Science (WoS).

Applicability of the ROR methodology to disasters of natural origin: In the case of natural disasters, the ROR methodology also showed its application potential: forecasting of Hurricane Irma; modeling of cold fronts and tropical cyclones for Cuba; prediction of the latitude and longitude of earthquakes at a global level, forecasting possibilities for earthquakes up to the year 2050 and forecasting of earthquakes in Haiti up to 2096. Results that made possible the publication of five scientific articles related to this topic, in prestigious and impact magazines, indexed in group 1 and 2 databases, and even some of them in the Web of Science (WoS).

ROR vs COVID-19 Methodology: The modeling of SARS-CoV-2, virus causing COVID-19 was carried out by means of the methodology of Regressive Objective Regression (ROR), both in Santa Clara municipality and Villa Clara province and for Cuba, which allowed us to make short, medium and long term forecasts according to COVID-19. On the other hand, with this methodology we were able to make prognoses of deaths and new cases with an advance of 105 days in Cuba, as well as prognoses for deaths, critical, severe, confirmed and new cases of COVID-19 in the Santa Clara municipality and at the national level; in addition to the application of this methodology to vaccination against COVID-19 in Cuba, and the comparison of the ROR methodology as a linear model with the non-linear Weibull model for COVID-19. Results that made possible the publication of ten scientific articles related to this topic, in prestigious and impact magazines, indexed in databases of groups 1 and 2, and even, several of them in the Web of Science (WoS).

CONCLUSION

The ROR methodology as a linear mathematical methodology has demonstrated potential and real capabilities of application in various fields and branches of science. The ROR methodology allows not only to mathematically model the larval densities of mosquitoes, as well as the population dynamics of mollusks, but goes beyond (possibility of modeling infectious entities of different etiologies (parasitic and viral), such as HIV/AIDS, Cholera, Chikungunya, Dengue, Influenza, and Acute Respiratory Infections (ARI), Acute Respiratory Infections (ARI), Acute Bronchial Asthma (CAAB), Zika, Angiostrongylosis, Fasciolosis, Malaria and even in the estimation of the longitude and area of the universe, monthly forecast of precipitation and extreme temperatures, forecast of extreme meteorological disturbances (cyclones and hurricanes); prediction of the

latitude and longitude of earthquakes, search for information on white noise, modeling of the Equivalent Effective Temperature (TEE) and Atmospheric Pressure (PA), and even the electricity consumption of a municipality, province and nation. The methodology in question is a contribution to the science of modeling and forecasting variables to know the future, as well as the impact of different variables, and what they contribute to an event or phenomenon; so it is a powerful tool to explain the phenomena of nature and society, since being universal, it can be applied anywhere in the universe.

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Bimetallic Nanoparticles – Expanding Grassroots in Medical Health Care through Enhanced Microbial Resistance

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ABSTRACT

Nanotechnology has spawned a slew of new research and application opportunities. Bimetallic nanoparticles offer certain advantages over monometallic nanoparticles in magnetic and optical properties which have utmost requirement in medicinal industry for therapeutics and drug delivery system. The advantage of using a green synthesis technique results in increased stability and enhancement of many physical and chemical characteristics. Green synthesis has the benefit of being able to replace existing physical and chemical processes. Different monometallic nanoparticles have already been tested. Researchers are working on even trimetallic nanoparticles in present scenario. In the present investigation research cum review article, we have randomly selected medicinal plants available in campus itself as lockdown conditions were prevalent all over the country. The bimetallic Cu-Zn nanoparticles were synthesized using mostly leaves as plant parts, from these selected plant materials. The Cu-Zn nanoparticles were confirmed by their synthesis, using visual color change along with UV visible spectroscopy techniques. The nanoparticles after confirmation were opted further for testing their efficacy against standard pathogens for plants viz *Terminalia*, *Tecoma* and *Solanum nigrum*. The results have been promising and significant giving zones bigger in size than 20 mm. The research is ongoing to reveal further the inherent potential of medicinal plants to act as alternative agents for antimicrobial potential.

KEY WORDS: NANOPARTICLES, ANTIMICROBIAL, PHYTOCHEMICALS, ZONE OF INHIBITION.

INTRODUCTION

From the birth of civilization, medicinal plants have been an integral element of human culture in the fight against illness. As a result, nanoparticles (NPs) can be described as particles with novel or better qualities to the bulk material from which they are made. It is among the most reliable fields in science these days. Numerous terminologies are used to describe nanotechnology; however the most notable often utilized terms are nanomaterial and nanoparticle, (Jeevarathinam et al., 2019, Umar et al., 2020). Nanoparticles have multifunctional properties and various applications with regards to different fields for example medication, energy and nutrition. The field of nanotechnology manages the particles present at nano-scale which will in general have interesting physio-chemical properties which render distinctive practical properties, (Abou-Okeil et al., 2012, Umar et al., 2020).

Progressed nano-biotechnology coupling biotechnology and nanotechnology has prompted the advancement of different

methods used to create nanoparticles. Nanotechnology is a multidisciplinary scientific domain that has been used in chemical, physical, biological, pharmacological and material science domains. Because of the vast range of applications for metallic nanoparticles has led to a rise in global investment in nanotechnology-based advancement and research. Physical and chemical nanoparticle synthesis methods have been used in the past and they are based on ion sputtering, hydrothermal synthesis, microemulsion, and sol-gel procedures (Armendariz et al., 2004, Antony et al., 2011 & Argueta-Figueroa et al., 2014).

Plant-based nanoparticle synthesis (Phytonanotechnology) opens up new possibilities in the realm of NP synthesis since it is a green, easy, stable, fast, and low-cost technique that employs biologically acceptable solvents and little or no toxic chemicals. Additional to these benefits, phytonanotechnology creates biocompatible nanoparticles, the procedures are adaptable, and no hazardous substances are used as reductants. The all-purpose solvent, water, is widely employed as a medium and a reductant. Since the synthesis may be carried out more safely and easily without the use of any chemicals, it is also known as a green synthesis strategy (Ashishie et al., 2018).

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The plant or portions of the plant can be extracted straight in a liquid medium in green synthesis that can be used in the formation of metal NPs as a reducing, capping or stabilizing agent. Numerous functional groups, such as phenolic or alcoholic groups and carboxylate groups, are used in the reducing, encapsulation, manufacturing, and preservation of NPs produced by the green synthesis process and so on. The limitations of microbial nanoparticles are readily defeated in green synthesis (Bai et al., 2006). Green synthesis tries to substitute harmful chemicals with biomolecules from plants that contain terpenoids, aldehydes, vitamins, alkaloids and polysaccharides that work as reducing, capping, and stabilizing agents in the production of desirable nanoparticles.

The characteristics of metals, as well as their applications, differ depending on the metal. Metal NPs have superior strength, malleability, and electrical-magnetic characteristics compared to their bulk counterparts. Magnetic metals (Fe, Co, Ni) (Beyth et al., 2015) and noble metals (Rh, Rd, Ag, Pd) (Brightson et al., 2010) are most commonly employed to make metal nanoparticles. The optical, electrical, plasmonic, thermal, magnetic, and catalytic characteristics of monometallic nanoparticles are altered by bimetallic nanoparticles synthesis in various forms, sizes, and compositions. Bimetallic nanoparticles because of the synergistic impact of two metals hence increasing their electronic effects, lattice stress, bifunctional effects, and ensemble effects all contribute to the utility and applicability (Balamurugan et al., 2016). Nanoparticles may be categorized according to source, size, and structure. They can be natural or manufactured on the ground of source; 0,1,2 or 3 dimensional in terms of dimension; liposomes, dendrimers, carbon-based, or metal-based depending on their structure (Balamurugan et al., 2016).

Fe, Co, and Ni are late transition elements that can be added to noble elements to reduce the cost of the raw components. Several attempts were undertaken in the recent decade for the green and affordable production of metallic nanoparticles with broad applicability in all fields of research. Green synthesis of metal/metal oxide nanoparticles, fetched a great deal of attention in the past few years due to its growing uses in a variety of disciplines, as well as its low chemical use and simple experimental procedures. It is more efficient and practical. As a result, multiple attempts were made to use plant extracts to synthesize nanoparticles of various metals such as silver, gold, palladium, zinc, copper, iron, cobalt, nickel, and so on.

Both primary and secondary metabolites included in extracts were shown to decrease metal ions and aggregate them into nanoparticles. Carbohydrates (simple sugars and polysaccharides), proteins, and lipids are primary metabolites, while alkaloids, glycosides, terpenes, tannins, flavonoids, and acetogenins are secondary metabolites (Chowdhury et al., 2018). Bimetallic or alloying nanoparticles consist of two monometallic nanoparticles in various proportions, the final architectures of nanomaterial show versatility in their properties. The synthesis of required structural composition of alloys mainly depends on the composition metals, the synthetic route and the reaction

conditions. Biosynthesis methods are more advantageous than any other classical procedures because of their accessibility to more biological substances.

The Biogenic synthesis utilizing organic concentrates are the expanding research consideration in nanotechnology, since it doesn't require a lot of additional energy and is unable to produce highly toxic chemical byproducts. Often times the chemical method for the synthesis of nanoparticles results in the formation of toxic chemical substances that are absorbed on the surface, and have destructive effects in the medical application. Due to production of these chemical products, green synthesis has come under consideration. The utilization of plant extracts rather than traditional chemical toxic chemicals with the same procedure of synthesis of nanoparticles is at present under huge examination. Plant nanoparticles are more advantageous than that of microbial nanoparticles as plant nanoparticles are more stable and take less time to extract metal ions.

Moreover, they are not required to be kept or grown in a sterilizing environment like that of the microorganism. Additionally, they may be appropriately expanded for huge synthesis of nanomaterials (Devi & Singh 2016) (Faiyas et al., 2010). In general, there exist four structural possibilities regarding the pattern of bimetallic nanoalloys: Core-shell structure, sub-clusters, statistically mixed and multiple shell alloys. Bimetallic nanoparticles are made up of two different metals that combine to exhibit physio-chemical properties unique to each metal. They are therefore significant both industrially and technologically.

Bimetallic nanoparticles are more significant when contrasted with those of monometallic nanoparticles because of the presence of additional degrees of freedom. By combining nanoparticle gold with other metals like silver, nickel, cobalt, and so on, its catalytic characteristics can be upgraded positively in the greater amount (Gopalakrishnan et al., 2017). Bimetallic nanoparticles have a more prominent surface zone which expands their adsorption properties and therefore acts as an effective catalyst as compared to those of monometallic nanoparticles. Bimetallic nanoparticles contain a few useful highlights for example, the capacity to work in a wide scope of pH, temperatures, attractive properties, etc.

Biogenic/Ecofriendly Synthesis and Confirmation

Techniques: Nanoparticle synthesis involves two key strategies: Top-down synthesis involves cutting through bulk materials to create nanoparticles, whereas bottom-up synthesis involves atoms accumulating into new compounds, which subsequently grow into bunches and produce nanoparticles (Hamid et al., 2013). A bottom-up method is used in the production of nanoparticles, where the two main reactions that take place are oxidation and reduction. The produced nanoparticles' content, size, morphology, and crystalline phase are inferred using Raman, UV-Visible, XRD, FT-IR, DLS, and EDS spectroscopy. Bimetallic nanoparticles show excellent resistance to Gram-positive pathogen e.g. *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative bacteria e.g. *Klebsiella pneumonia* and *Escherichia coli*.

When these nanoparticle search out to the bacterial cells, they pass through their cell wall and cell membrane and immediately connect with the bacterial cell's components including DNA, lysosomes, ribosomes and enzymes, leading to osmotic damage, diverse changes, variations in cellular uptake, problems with osmo-regulation, etc. Nanoparticles are more effective against Gram-positive pathogen because their Cell wall is composed of a peptidoglycan layer and teichoic acid consisting of ultra-small pores through which the nanoparticles can easily pass and hence causes damage to the cell wall resulting in the death of bacteria but the cell wall of Gram-negative pathogens is made up of lipopolysaccharides that only allow macromolecules to pass through it.

An emerging area of nanobiotechnology called "green synthesis" has advantages over conventional chemical and physical procedures in terms of both economy and the environment. Chemicals that are harmless, eco-friendly, and safe are used in this process. Plant extracts (Huang et al., 2007), cyclodextrin (Hyeon 2003), chitosan (Jamdagni 2018) and a variety of other natural resources have been investigated in the manufacturing of metal oxide nanoparticulate. The utilization of plant extracts in the green production of metal oxide nanoparticles has received a lot of interest as a simple method (Huang et al., 2007) & (Jeevarathinam et al., 2019). Metal oxide nanoparticles have been created synthetically from plant extracts by a number of researchers, (Huang et al., 2007) along with his coworkers were one of many who looked into the extracellular creation of Zn nanoparticles from seaweed. The authors hypothesized that fucoidan, a pigment that dissolves in water found in *Sargassum myriocystum* may reduce and stabilize the synthesis of Zn nanoparticles. Furthermore, because *S. myriocystum* is available throughout the year, it is possible to simply scale up the synthesis of Zn nanoparticles (Khatami et al., 2018 Jeevarathinam et al., 2019, Karupannan et al., 2020).

Jamdagni and associates in 2016 and Sithara and her co-researchers in 2017 improved the conditions for synthesizing Zn nanoparticles and Ag nanoparticles, respectively. Sithara and her coworkers optimized the concentration of the precursor, silver nitrate, the amount of plant extract and the temperature of the solution. The optimal concentration, volume, and temperature according to the results were 1.75 mM, 0.5 mL, and 500C, respectively. They also discovered that adding plant extract to silver nitrate changed the hue from colorless to brown, indicating that Ag nanoparticles were formed.

The absorption of the spectrum at 300-500 nm was measured with ultraviolet visible light to validate the manufacturing of nanoparticles. A band at 425 nm indicated the existence of Ag nanoparticles (Huang et al., 2007). The temperature has an essential influence in regulating the aspect ratio and relative numbers of gold nanotriangles and spherical nanoparticles during biogenesis. The form, size, and optical characteristics of anisotropic nanoparticles may be fine-tuned using temperature fluctuations in reaction conditions (Kitchens et al., 2005).

Magnolia kobus and *Diopyros kaki* leaf extracts were quickly transformed to gold nanoparticles at a reaction temperature of 95 °C, with over 90% of the extracts undergoing this transformation, implying reaction speeds greater or equivalent to those of chemical nanoparticle synthesis (Kumar et al., 2020). At higher reaction temperatures the size of gold nanoparticles was found to rise which was explained by an increase in micelle fusion efficiency, which dissipates super saturation (Kuppusamy et al., 2016). The size of nanoparticles is strongly influenced by the medium's pH. In *Avena sativa* (Logeswari et al., 2015) the size of gold nanoparticles was regulated by changing the media's pH. When the co-precipitation technique was used, the reaction process for generation of magnetite micro-particles was discovered as pH-dependent (Malapermal 2015, Kumar et al., 2020).

Other parameters, in addition to pH and temperature have a role in nanoparticle production. During hydrothermal synthesis using the Schikorr reaction (Malapermal et al., 2015) the size and crystallinity of 11 magnetite nanoparticles were shown to grow with increasing molar ratios of ferric/ferrous ions. With rising NaCl concentrations the size of gold nanoparticles shrinks (size ranges, 5-16 nm) than those made without the use of NaCl (size ranges 11-32 nm) (Mazhar et al., 2017).

Optical absorption spectroscopic technique Manjari et al., (2020) showed that the band gap energy in ZnS samples decreases with increasing dopant concentration. Nanoparticle production in plants is influenced by chloride, bromide, and iodide. Chloride enhances nanotriangles development, whereas iodide disrupts nano-triangles structure and leads to the creation of aggregated spherical nanoparticles (Minal & Prakash 2016). The production of diamond-shaped copper nanoparticles is caused by the chloride ion (Mizutani et al., 2008). Sun-dried *Cinnamomum camphora* leaf biomass generates triangular or spherical gold nanoparticles as well as silver nanoparticles (55-80 nm) when aqueous silver or gold precursors are kept at room temperature. The relative potential of protecting and reducing biomolecules from leaf extracts might explain by significant shape variation between gold and silver nanoparticles. The polyol and aqueous soluble heterocyclic compounds were primarily responsible for the reduction of silver ions or chloroaurate ions (Mohamad et al., 2011).

Figure 1: Visual confirmation of Bimetallic Cu-Zn nanoparticles from leaf extracts of *Terminalia*(a) ,*Tecoma* (b) and c) *Solanum*



The visible color change of blue copper sulfate was used to confirm the presence of bimetallic Cu-Zn nanoparticles and colorless zinc nitrate solution to greenish colored solution on reduction using plant leaf extracts and as plant part

offering dual benefit of capping and reducing effects (Fig-1). While UV visible spectrophotometer revealed two peaks one at 404nm and other at 408nm confirming nanoparticle synthesis. India is flooded with such bio resources and sacred plants like Tulsi, Baelpatra and Kadamba having medicinal and scientific logistics which need to furnish by developing drugs using ancient knowledge and Ayurveda medicinal flora.

Nanoparticles as Antimicrobial agents: There are two sorts of applications for metallic nanoparticles that have been investigated; biological and non-biological applications. Nanoparticles have been exploited as antibacterial, antifungal, antiviral, anticancer, antidiabetic, and antioxidant properties in biological applications (Mokhena & Luyt 2017), (Mostaghnia et al., 2017) & Muralidharan et al., 2011). Harmful dyes including methylene blue, 4-nitrophenol and its derivatives utilized as pesticides, and usage in dye-sensitive solar cells (DSSCs) can all be photocatalyzed (Nava et al., 2017) and are non-biological uses. In the coming decade, numerous applications will result from the quick advancement in the production of bimetallic nanoparticles with distinctive physical, chemical and biological features. When two metals with different qualities are combined in a single nanosystem, it reveals that they can be used in a range of platforms thanks to its electrical, biological, thermal, chemical and mechanical properties (Nava et al., 2017).

Antimicrobial properties of bimetallic nanoparticles can supplement antibiotics in the fight against microorganisms. Also, antibacterial efficacy of Au-Ag bimetallic nanoparticles generated from *Ocimum basilicum* (Basil) flower and leaf extracts against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and other bacteria has been reported by researchers (Nazeruddin et al., 2014). Ag-Cu bimetallic nanoparticles have been found to have antimicrobial effects against the gram-positive *Bacillus subtilis*. As a result, antibacterial medicines can be created using their combined actions with antibiotics and sulfa drugs (PannareeSrinoi et al., 2018).

These nanoparticles can obstruct bacterial development by disrupting membranes or creating reactive oxygen species (ROS) which induce DNA degradation and obstruct mechanisms for protein activity (Porter & Youtie 2009). Ag-Au metallic produced from *Gracilaria* sp. show

antimicrobial action against *Staphylococcus aureus* and *Klebsiella* (Joerger et al., 2000). Antimicrobial action of Ag doped ZnO nanoparticles against *Staphylococcus aureus* and *Bacillus subtilis* by changing the MIC (Minimum Inhibition Concentration) particularly for *S. aureus* has been reported (Ramakritinan et al., 2013).

Cu-Ni bimetallic nanoparticles have been found to exhibit bacteriostatic properties against microbes like *E. coli*, *Staphylococcus aureus*, and *Staphylococcus mutans* (Rane et al., 2018). Antifungal and antibacterial properties of Fe-Ag magnetic bimetallic nanoparticles have been demonstrated against a variety of harmful microorganisms. These antibacterial bimetallic nanoparticles might be employed in nanomedicine to create nanodrugs against human infections to fill in the gaps where antibiotics have failed to provide favorable outcomes.

RESULTS AND DISCUSSION

Antimicrobial Efficacy of Terminalia-Mediated Bimetallic Cu-Zn Nanoparticles: *Terminalia chebula* Retz. family *Combretaceae* clearly depicts the applicability of its different plant parts in several ailments. It is found in Uttar Pradesh, Bengal, and North India's forests and is very frequent in existence in Southern part of India. The plant is a huge tree, is found in China and other tropical and subtropical regions of Asia. Ailments like cough, gastroenteritis, diarrhea, fever, skin disease, urinary tract infection are cured using harad by Tribal people of Karnataka and TamilNadu (Rani et al., 2018). The plant parts are boon to nano-researchers as all type of nanoparticles can be synthesized using this plant as sole source.

We recommend researcher to work on different nanoparticles synthesis using terminalia as sole plant source as initial color development was observed in our preliminary experiments for all types (Cu, Zn, Ag, Ag-Cu and Cu-Zn) of nanoparticle synthesis. ZnO and silver nanoparticles have extensive application in coating, painting industries, personal health care products, spin electronics, chemical sensors, light emitters and transparent electronics. Bimetallic Cu-Zn nanoparticles have more efficient systems to inhibit resistant strain of bacteria owing to better optical and magnetic characteristics which in turn is due to merging two metallic particles together. The zone size reported using bimetallic nanoparticles was above 20mm in size as compared to monometallic nanoparticles (Table-1), (Fig-2).

Table 1. Inhibition zone diameters (in mm) of copper-Zinc nanoparticles of *T. chebula* (leaf) against different pathogens.

Sr. no.	Concentration of extracts(µl)	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
1.	50	23	29	19	18
2.	75	28	30	22	25
3.	100	29	32	25	27
PE	100	-	-	-	-

Figure 2: Zones of inhibition using Bimetallic Cu-Zn nanoparticles from Harad leaf plant extracts tested against four pathogens. a) *P. aeruginosa* b) *E. coli*. c) *S. aureus* d) *B. subtilis*.



Three volumes of extract were tested against four standard pathogens viz. *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. Significant zone of inhibition were observed as shown in Table-1 at all three volumes of extracts. The bimetallic nanoparticles resulted in zones of inhibition of size 23mm, 28mm and 29mm at three different volumes of 50, 75 and 100µl respectively while aqueous extract failed to generate any zone of inhibition. Similarly zones of size 29mm, 30mm and 32 mm were observed at three different volume of extract of 50, 75 and 100µl respectively. The zones show linear correlation to the volume concentrations

used and validate the increase in volume extract resulting in increase in zone size even when there is minor difference of 25µl between different volumes of extract against *E. coli* a gram negative bacteria.

While testing against a gram negative strain *P. aeruginosa* significant zone of inhibition but lesser in diameter as compared to *E. coli* were observed resulting in zones of 19mm, 22mm and 25mm at three different volumes of 50, 75 and 100µl respectively. Similarly when tested against *B. subtilis* particular zones of 18mm, 25mm and 27mm were observed as shown in figure-7 clearly at three different volumes of extract of 50, 75 and 100µl respectively. In all cases there is positive correlation between zone size and volume of extract used. No contradiction was observed and further results obtained are promising in futuristic alternative to be used as antimicrobial agents. The bi functional nanoparticles exhibit cumulative effect which in turn is due to alteration in the geometric pattern and electronic properties resulting in enhanced activities as compared to monometallic nanoparticle synthesis (Patil & Kumbhar 2017). The atoms of two metals have parts of active catalytic sites which increase adsorption for more variable reactants or variable intermediates.

Antimicrobial Efficacy of Tecoma-Mediated Bimetallic Cu-Zn Nanoparticles: *Tecoma stans* family *Bignoniaceae* ornamental plant is The Bahamas' national flower. It is between 5-7.6 meters tall and is a perennial blooming shrub or small tree. Three volumes of extract were tested against four standard pathogens viz. *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*. A significant zone of inhibition was observed as shown in Table 2 at all three volumes of extracts.

Table2. Inhibition zone diameters (in mm) of copper-Zinc nanoparticles of *T. stans* (leaf) against different pathogens.

Sr. no.	Concentration of extracts(µl)	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>
1.	50	25	25	17	24
2.	75	31	33	24	28
3.	100	33	34	27	28
PE(Aq)	100	--	-	-	-
Cu-Zn	100	21			

The bimetallic nanoparticles synthesized using *Tecoma* leaf resulted in zones of inhibition of size 23mm, 28mm and 29mm at three different volumes of 50, 75 and 100µl respectively, while aqueous extract failed to generate any zone of inhibition. Similarly zones of size 25mm, 31mm and 33 mm were observed at three different volume of extract of 50, 75 and 100µl respectively. The zones show linear correlation to the volume concentrations used and validate the increase in volume extract resulting in increase in zone size even when there is minor difference of 25µl between different volumes of extract against *S. aureus* a gram positive bacteria.

While testing against a gram negative strain *P. aeruginosa* significant zone of inhibition but lesser in diameter as compared to *E. coli* and *S. aureus* were observed resulting in zones of 17mm, 24mm and 27mm at three different volumes of 50, 75 and 100µl respectively. Similarly when tested against *B. subtilis* particular zones of 24mm, 28mm and 28mm were observed as shown in Table -2 clearly at three different volumes of extract of 50, 75 and 100µl respectively. Similarly zones of size 25mm, 33mm and 34 mm were observed at three different volume of extract of 50, 75 and 100µl respectively when tested against *E. coli* a gram negative bacteria. In all cases there is positive

correlation between zone size and volume of extract used. No contradiction was observed and further results obtained are promising in futuristic alternative to be used as antimicrobial agents.

Antimicrobial Efficacy of Solanum Mediated Bimetallic Cu-Zn Nanoparticles: In the present study the zinc nanoparticles manufactured utilizing leaves of *Solanum nigrum* resulted in significant zones of inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Three concentrations of Cu-Zn nanoparticles were used, which are 50, 100 and 150 µl. Zone size of 19mm to 29mm was reported against *Bacillus subtilis*, while a minimum zone of 23mm and a maximum of 28mm were reported for *Staphylococcus aureus*. Against *Escherichia coli* and *Pseudomonas aeruginosa* the range of zone of inhibition (ZOI) were in the range of 19mm to 25mm and 24m to 26mm respectively.

With *Candida albicans* it can be concluded that the three concentrations yielded fairly nearing ZOI with a minimum being 26mm and a maximum at 28mm as shown in Table 3 and Fig 3. (Hamid et al.,2013) synthesized gold and silver-gold bimetallic nanoparticles via green route and aqueous extracts of sago pondweed resulting in spherical-shaped nanoparticles along with two different shapes of hexagon and nano triangles. The nanoparticles were dependant on pH and proteins and flavonoids present in phytochemical analysis proved to be major factor for nanoparticle synthesis. (Minala& Prakasha2019) reported larvicidal activity using *Azadirachta indica* leaf extracts.

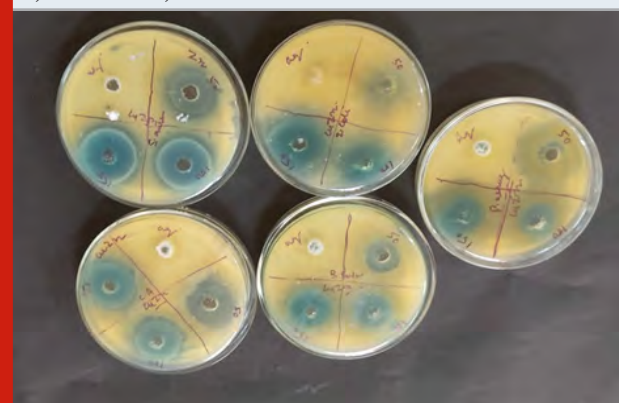
Table 3. Inhibition zone diameters (in mm) of copper-Zinc nanoparticles of Solanum (leaf) against different pathogens.

Pathogen	Volume of Extract (µl)	Cu-Zn Np from leaves (mm)
<i>Bacillus subtilis</i>	50	19
	100	25
	150	29
<i>Staphylococcus aureus</i>	50	23
	100	27
	150	28
<i>Escherichia coli</i>	50	19
	100	22
	150	25
<i>Pseudomonas aeruginosa</i>	50	24
	100	25
	150	26
<i>Candida albicans</i>	50	26
	100	26
	150	28

Finally, bimetallic nanoparticles outperformed monometallic nanoparticles in terms of larvicidal activity. (Devi & Singh,

2016) used ascorbic acid as a new green approach as a reducing and capping agent for the reduction of metal salts for the synthesis of monometallic copper and zinc along with bimetallic nanoparticle synthesis. Cu-Zn and Cu-Ni resulted in particle sizes of 43.47, 38.4 and 43.5nm for Cu, Cu-Zn and Cu-Ni nanoparticles respectively. The UV spectroscopy revealed that the solution is not just a mixture of solutions but is an alloy. Bimetallic nanoparticles were more potent in photo-degradation Cu-Zn(80.75) and Cu-Ni (80.4%) of methyl orange in 60 minutes as compared to monometallic nanoparticles (69.5%) due to bifunctional effects. (Mostaghni et al., 2017) manufactured Cobalt Iron Oxide powder utilizing *Chenopodium album* leaf extract.

Figure 3: Zones of inhibition using Bimetallic Cu-Zn nanoparticles from Solanum leaf plant extracts tested against pathogens. a) *P. aeruginosa* b) *E. coli*. c) *S. aureus* d) *B. subtilis* e) *Candida Albicans*



X-ray diffraction techniques were used to know the structure of the synthesized material. The photocatalyst that was created was used to photodegrade methyl orange as a useful model contaminant. According to the findings, CoFe_2O_4 was highly effective at destroying germs when exposed to UV light, with a degrading ratio of 100 % after three hours of exposure. (Rani et al., 2018) exploited four plants namely *Chenopodium album* (JungliBathua), *Mesua ferrea* (Nagkesar), *Syzygiumcumini* (Jamun), and *Cassia fistulav* (Amaltas) to manufacture silver nanoparticles and their antibacterial function was tested against *Alternaria*, *Excrotium*, and *Fusarium oxysporum*. These silver bio-nano particles were tested with phytopathogenic fungus for sustainable crop production. (Khatami et al.,2018) synthesized silver and zinc nanoparticles using *Prosopisfracta* and *Cofea arabica*.

Standard confirmation via UV visible spectroscopy, XRD and SEM results in an average nanoparticle size 16 and 26nm respectively. further the nanoparticle were tested against *Acinetobacter baumannii* and *P. aeruginosa* culture and tested bandages which were dipped /impregnated with ,silver,zinc and also in mixed solution silver and zinc in equal proportion were more efficient in healing. bandages dipped in both solutions were more efficient in curing, diabetes or burn injuries are particularly susceptible to infections. (Ashishie et al.,2018) synthesized silver monometallic and copper-silver bimetallic nanoparticles using *Kigelia africana* fruit extracts.

Aqueous plant extracts of fruits were characterized using standard techniques and methodology. The SEM images showed uniform spherical silver nanoparticles while silver copper was anisotropic in nature. The average size was reported as 10nm. The nanoparticles exhibit strong inhibitory capability against both gram + and gram - strain tested. It inhibited *K. pneumonia* and *P. aeruginosa* resulting in zones of 23 and 25mm in size. Using silver nanoparticles while bimetallic results in zones of 27mm against *S. aureus*. XRD analysis revealed crystalline particles. (Umar et al., 2020) investigated the green synthesis of reduced graphene oxide nanoparticles utilizing *Chenopodium album* (Bathua in Hindi) a commonly found weed plant. Reduced graphene oxide (RGOX) from graphene oxide (GOX) was devised using vegetable extract as a reducing agent. When it came to antibacterial performance against both Gram-positive and Gram-negative microbes as well as antibiofilm action, the agar diffusion test outperformed GOX.

Nanoparticles, particularly metallic nanoparticles have piqued the curiosity of a variety of organizations and professions including electronics, photonics, medicine and agriculture. Recent analysis of the manufacture of metallic nanoparticles utilizing biological organisms has been covered in this study. However, due to the variety of living organisms, from bacteria to plants, the majority of this issue is still understudied and unknown. The creation of nanoparticles from biological sources has the potential to open up new possibilities for the production of distinctive materials that are ecologically friendly, affordable, stable, and free of hazardous substances. Modern chemical and physical procedures usually involve risky compounds that can cause environmental toxicity and carcinogenic effects.

This green chemistry technique, which uses biological organisms, contrasts dramatically with such processes. Although many biological sources have been used to create nanoparticles, employing plants offers a straightforward, safe, non-toxic, and robust method that does not necessitate the additional culture preparation or isolation steps that are frequently needed for microbes and fungi-based approaches. Plant extracts are affordable, readily scaled up, and environmentally acceptable when used to synthesize nanoparticles of a specific size, form, and composition. Plant-derived nanoparticles have the possibility of replacing several currently used nanoparticle-based medical treatments, including fluorescent labeling in assay methods, therapeutically drug delivery, tumor killing by overheating (hyperthermia), and antimicrobial agents in bandages. Plant-derived nanoparticles, on the other hand, have the ability to transport anti-microbial chemicals as pesticides in agricultural crops. Furthermore, Agricultural crop wastes and wastes from the food sector are a great source of plant-based biochemicals that can be used to create metallic nanoparticles and other similar products.

Regardless of the ecological benefits of using eco-friendly chemistry-based biological production over conventional approaches, as mentioned in this article, some problems remain unresolved, such as the consistent properties of nanoparticles, reproducibility of the synthesis process, and

comprehension of the factors underlying the production of metallic nanoparticles via living creatures. Nanoparticle production processes differ amongst plant species when it comes to plant extracts. As a result, to study and fully appreciate the scenario where plants are dependent, research is required. Nanomedicine has a strong impact being potential agents in disease diagnosis and treatment, developing surgical devices and other commercial health products.

Green synthesis offers a remarkable progression over both physical and chemical approaches since it is affordable, environmentally friendly, and can be scaled up successfully for large-scale synthesis. Synthesis from plant extracts has many advantages, including sanitary workplace conditions, environmental and health protection, low waste, and extremely reliable products. Bimetallic nanoparticles produced using a green method has several uses in nanotechnology that are significant.

CONCLUSION

Nanotechnology has conquered almost all fields ranging from agriculture to tissue engineering. Utilizing plants for the green production of nanoparticles includes economic and environmental conservation. Thus, biosynthesized nanoparticles could be used in toothpaste, mouth wash and mouth fresheners to make them more effective. The antibacterial activity of clinical cotton bandages dipped in nanoparticle solutions showed good antimicrobial activities which open the door for antimicrobial bandages, tissues and diapers for babies in the future.

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IOT Based Paraplegia Patient Communication Device Using Smart Glove

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ABSTRACT

Paraplegia is a paralysis that affects the legs and often seriously affects the lower half of a human body. Paraplegia happens whilst there may be damage underneath the neck. The maximum common motive is trauma, such as sports injury or vehicle accident. other causes are stroke, spine tumors, together with cancers. This paper provides a novel design to enhance the conversation of paralysis sufferers. The device is a clever glove that is used to seize hand gestures and convert them into verbal commands. The glove is geared up with sensors and processors that discover the hand gestures and convert them into indicators. The tool is designed to be simple and user-friendly, allowing patients to quickly adapt to its use. The tool is likewise designed to be low-cost and handy, permitting more paralysis patients to use it. The device is tested and evaluated for its performance, and the outcomes display that it may accurately capture and interpret the hand gestures of the sufferers. In most cases paralyzed affected person (like Monoplegia, Hemiplegia, Paraplegia) aren't capable of interact or communicate with others or their caretaker. This net of factors primarily based clever glove facilitates the affected person to convey their needs to the caretakers. It measures pulse, temperature, blood pressure and gyro. In gyroscope sensor there is a message, based at the route or motion in which it can be conveyed thru show and audio. it is able to screen thru internet and smart telephones. but it can not be used for tetraplegia patient. It is concluded that in quadriplegia as patients can not move their body, if in future we can able to read the neuron signals of the patients through sensor or any device and then we can able to understand what they need or what they want. Consequently the system can be able to display or hear through audio which can be used according to the need, updating into neuro reading sensors which are economically viable

KEY WORDS: SMART GLOVE, HEART RATE SENSING, GESTURE, TEMPERATURE, GSM, SPEAKER, DISPLAY.

INTRODUCTION

According to a survey conducted by WHO in Every year, around the world, between 250 000 and 500 000 people suffer a spinal cord injury (SCI). The majority of spinal cord injuries are due to preventable causes such as road traffic crashes, falls or violence. Males are most at risk in young adulthood (20-29 years) and older age (70+). Females are most at risk in adolescence (15-19) and older age (60+). Studies report male-to-female ratios of at least 2:1 among adults, sometimes much higher. This type of patient is can't able communicate with others like normal people like drinking, switching on fan. After analysing this problem, we propose a machine known as smart glove with a view to combine a function to serve the disabled individuals and assist them to speak freely with the open world. They can send a message to their caretaker; they can call to their doctor for any assistant. Consequently, at the same time as

these controls are beneficial for a generally abled character, they may be not very beneficial for the bodily handicapped or especially, the aged people, who do now not have the capability to use sufficient strain to move their hand.

Workers have proposed a first-generation prototype glove, where the glove converts gesture to text, just by moving the fingers. The blind person cannot walk casually in busy roads, the glove has an in-built obstacle sensor. The buzzer will ring when an object comes in front of the person (Nair, 2020, Kumar 2021). This is a unique device which incorporates multiple sensors, making it capable to do multiple tasks. Proposed a majorly useful for deaf and dumb, paralyzed patient to monitor their mental health by displaying the output commands in android application with vocal output in speaker. In case of emergency, GSM will send alert message to the respective person (Nair, 2020, Kumar 2021).

Proposed devices are mostly used in ICU to monitor the patient health in real-time, where the data is uploaded to the webserver and to the mobile application in which the

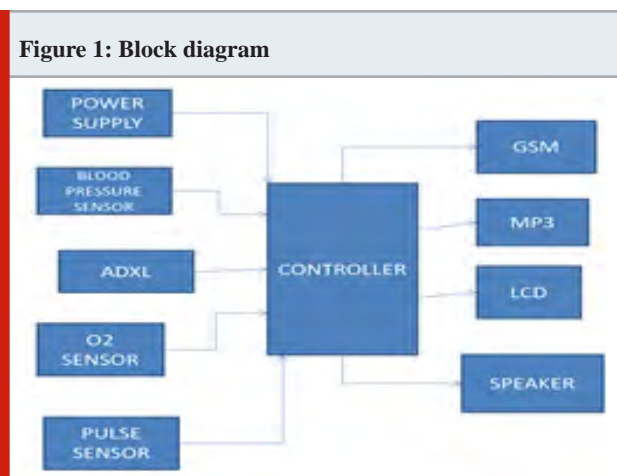
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output can be taken by doctor using the application (Babar & Rahman 2021). The doctor can categorize the patients by their status using an "IoT Based Patient Checking systems like framework utilizing TCP/IP convention, where the patient wellbeing is constantly followed even from the remote end. Also, with the assistance of set off notice SMS and call we can help the patient by taking vital choices right away as suggested by Sujin et al., (2021).

Furthermore, empowering the specialist to give medical aid remotely by observing the patient medical issue remotely which is made accessible internationally proposed a real time ECG signal was displayed on the web and storing it in the cloud server. In this they use a high-speed network so, there are no packages loss and package error during transmission. The doctor or nurse can observe the ECG signal of any patient at any place through the computer or smartphone without going to wards. This reduces the patient travelling time from rural or suburban areas. It also indicates the abnormal reading from the ECG signal (Yew et al., 2020, Fraiwan et al., 2018).

MATERIAL AND METHODS

ADXL is an acceleration detection sensor. It works under the principle of capacitive. It works when the acceleration is applied to the sensor, the capacitive inside the sensor changes. This change in capacitance is then used to measure the acceleration of the object. PIC is a family of microcontrollers. It is manufacture by microchip technologies. It comes under PIC1650(8-bit microcontroller).PIC refers to Peripheral interface controller. PIC is a one chip microcontroller (it is used to solve small task in a simple and cheap way). It has 40 pins. It has 33 I/O pins. Liquid crystal display (LCD) is a flat display technology. It is used to display the information. GSM (Worldwide Framework for Portable correspondence) is a computerized versatile organization that is broadly involved by cell phone clients in Europe and different regions of the planet. GSM utilizes a variety of time division various access (TDMA) and is the most broadly utilized of the three computerized remote communication innovations: TDMA, GSM and code-division different access (CDMA) (Athota 2020, Chouhan 2014, Rao 2013).



GSM digitizes and packs information, then sends it down a channel with two different floods of client information, each time permitting space. It works at either the 900 megahertz (MHz) or 1,800 MHz recurrence band. Mp3 a packed computerized sound record (Athota 2020, Chouhan 2014, Rao 2013). A player stores these records on a memory chip with the goal that you can play it back at your relaxation. Contains a little hard plate drive, which can store enormous amounts of information. MP3s normally contain music, however the configuration is additionally utilized for general sound documents, for example, book recordings and webcasts. Audio system work through changing electricity into mechanical energy (motion). The mechanical energy compresses air and converts the motion into sound power or sound pressure stage. while an electric current is sent via a coil of cord, it induces a magnetic field (Ganeson 2016, Tmaura 2013).

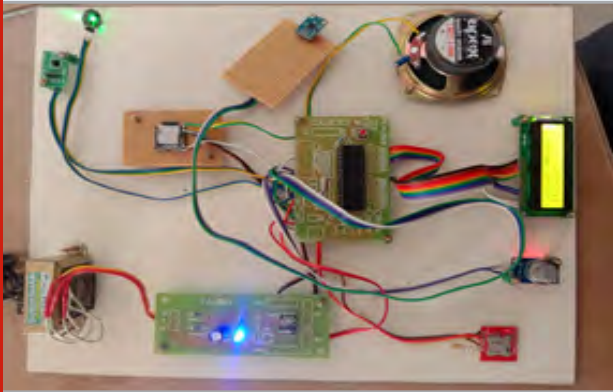
1. Create a design plan: The first step in creating a paralysis patient communicating device using a smart glove is to come up with a design plan. The plan should include the type of components to be used, such as the accelerometer (ADXL), PIC controller, GSM, LCD, MP3 and speaker, as well as the overall design of the device.
2. Implement the design: Once the design plan is complete, the next step is to implement the design. This involves connecting the components to each other and programming the device with the necessary software. Considerations should be made for the patient's comfort, as well as the device's ability to withstand being handled by the patient.
3. Test the device: After the device is assembled, it should be tested to make sure that it is working as expected. This includes testing its ability to detect the patient's movements, as well as its ability to communicate with the GSM and MP3.
4. Evaluate the device: Once the device is tested, it should be evaluated to ensure that it is working properly and providing the intended results. This includes testing the device for accuracy and reliability.
5. Make necessary modifications: If necessary, modifications should be made to the device to improve its performance. This could include adding additional sensors or programming more complex algorithms.
6. Deploy the device: After all of the necessary tests and modifications are complete, the device should be deployed to the patient. This involves setting up the device and training the patient on how to use it.
7. Monitor the device: Once the device is deployed, it should be monitored to make sure that it is working properly and providing the desired results. This could include collecting data on the patient's movements and responses to the device.

RESULTS AND DISCUSSION

The Paralysis Patient Communicating Device using Smart Glove is a revolutionary device that can help people with paralysis to communicate with others. It is equipped with ADXL, PIC Controller, GSM, LCD, MP3 and Speaker which are used to detect user's hand gestures and convert

them into commands. This device can help the user to type messages, play music, and even make calls. The device can be used as a communication tool to bridge the gap between the user and the other person. This device can be a great help to those who are suffering from paralysis and cannot communicate verbally.

Figure 2: Hardware Kit



**Simulation Output:
Figure 3: Basic Connection**

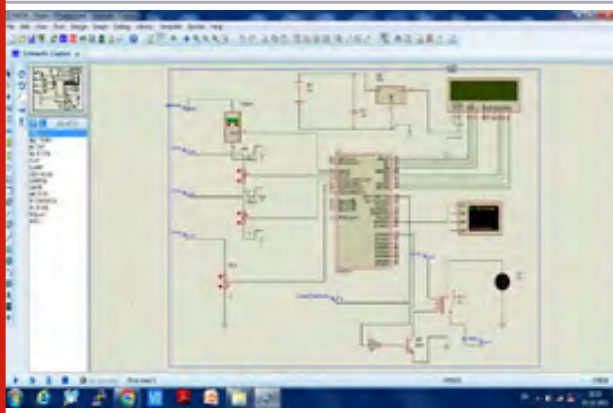


Figure 4: Command 1 (up)

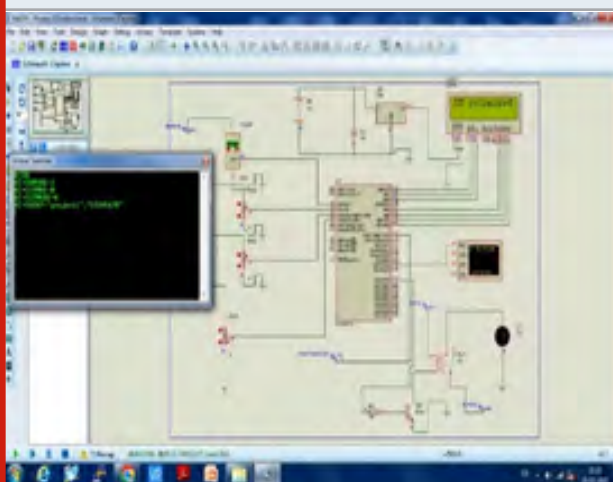


Figure 5: Command 2 (down)

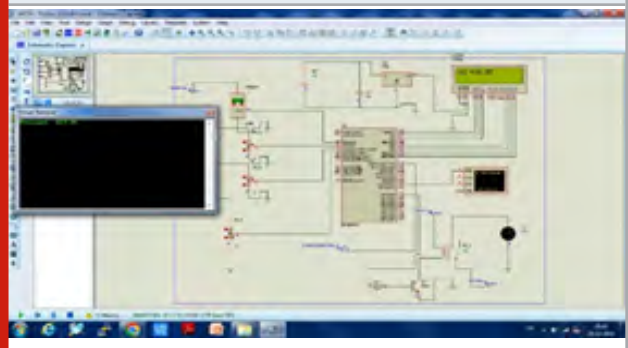


Figure 6: Pressure Sensor Output

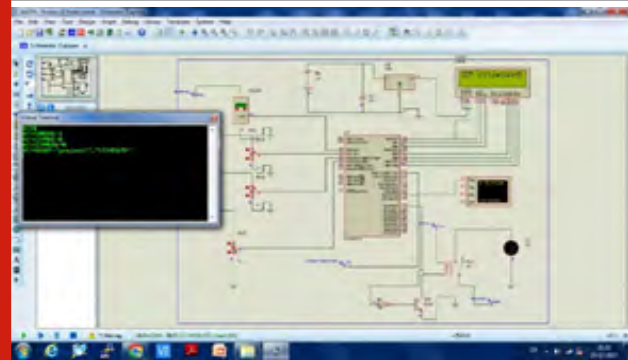


Figure 7: Heart Beat Rate

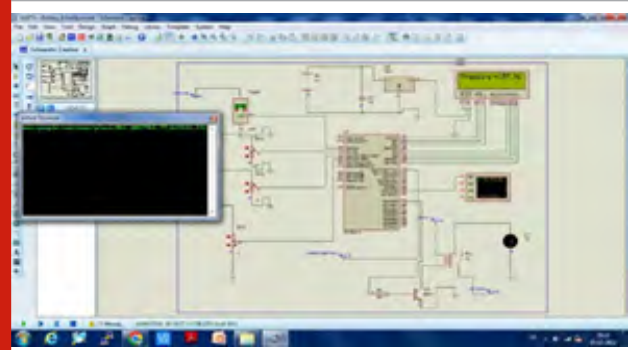
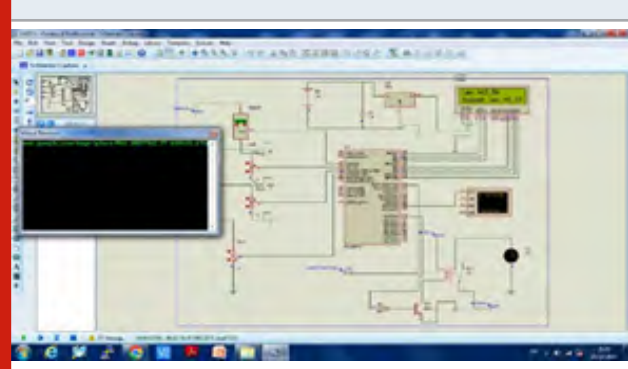


Figure 8: GSM



CONCLUSION

It is concluded that in quadriplegia as patients can not move their body, if in future we can able to read the neuron signals of the patients through sensor or any device and then we can able to understand what they need or what they want. Consequently the system can be able to display or hear through audio which can be used according to the need, updating into neuro reading sensors which are economically viable.

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Place : Bhopal

Bioscience Biotechnology Research Communications

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