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FEN, ZİRAAT ve SAĞLIK BİLİMLERİ
BİLDİRİLER KİTABI

4

PROCEEDINGS BOOK
SCIENCE, AGRICULTURE and HEALTH SCİENCES

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İÇİNDEKİLER

Extraction Of Triterpenoids from Ziziphus Jujube and Ganoderma Lucidum

Abdul Rehman[1]

Abstract

I study the extraction of triterpenoids from jujube and Ganoderma lucidum which is the most important component in Ziziphus Jujube and Ganoderma Lucidum. This study determined the optimal process for ultrasound-assisted extraction (UAE) of total triterpenoids from jujube fruit using response surface methodology (RSM). The optimal conditions were as follows: temperature of 55.14 ◦C, the ethanol concentration of 86.57%, time of 34.41 min, and the liquid-to-solid ratio of 39.33 mL/g. The triterpenoid yield was 19.21 ± 0.25 mg/g under optimal conditions. The triterpenoid profiles and antioxidant activity were further analysed. In Ganoderma lucidum, optimized extracts were fully characterized in terms of individual triterpenoids by HPLC-DAD-ESI/MS. With its beautiful legends, G. lucidum was believed by the ancient people to cure many kinds of diseases, and it was considered as an elixir that could revive the dead. In modern times, the mystery of G. lucidum arose the interest of scientists all over the world, and the publications and patents of G. lucidum have increased every year. The results provide important guidance for quality evaluation and pharmaceutical application.

Keywords: Ultrasound-Assisted Extraction, Ganoderma Lucidum, Triterpenoids, Ziziphus Jujube.

Introduction:

The consumption of fruits and vegetables is very important in human nutrition. They provide nutrients and non-nutritive constituents with significant biological activities, thus contributing to a healthy diet, with a reduction of disease risk. Based on scientific data, nutritionists suggest that increasing fruit and vegetable consumption is one of the best strategies to decrease the burden of several chronic diseases. Scientific evidence showed that higher consumption of

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fruits and vegetables is associated with a lower risk of all-cause mortality, particularly cardiovascular mortality [1]. The search for a healthier way of life has generated in the last two decades rising attention for the obtaining of pentacyclic triterpenoids, plant bioactive compounds that are increasingly demanded by the markets because they could be introduced in new functional foods, drugs, or cosmetics [2]. Jujube (*Ziziphus jujuba Mill.*), belonging to the Rhamnaceae family, is widespread in Asia, Europe, and America. In China, jujube has been cultivated for 4000 years and there are more than 700 cultivars of fruits. More than four million tons of jujube fruits are harvested in China per year, which represents 90% of the total yield globally. The fruit has been commonly used in Traditional Chinese Medicine (TMC) for its various pharmacological activities, such as its anticancer, antiepileptic, anti-inflammatory, anti-insomnia, and neuroprotective effects [3]. In general, the beneficial effects of health are derived from a variety of bioactive compounds, such as triterpenes, alkaloids, flavonoids, and polysaccharides. Triterpenes, belonging to the Phytosterol family (Figure. 1), are naturally occurring bioactive components that are commonly found in cereals and vegetables. Modern studies have shown that triterpenes and triterpenic acids, derivatives of pentacyclic triterpenes, have a variety of biological effects, such as antioxidative,anti-inflammatory, anticancer.

hepatoprotective, and anti-microbial activities, combined with low toxicity. Triterpene acids in jujube fruit have been demonstrated to be a group of major bioactive compounds [4].

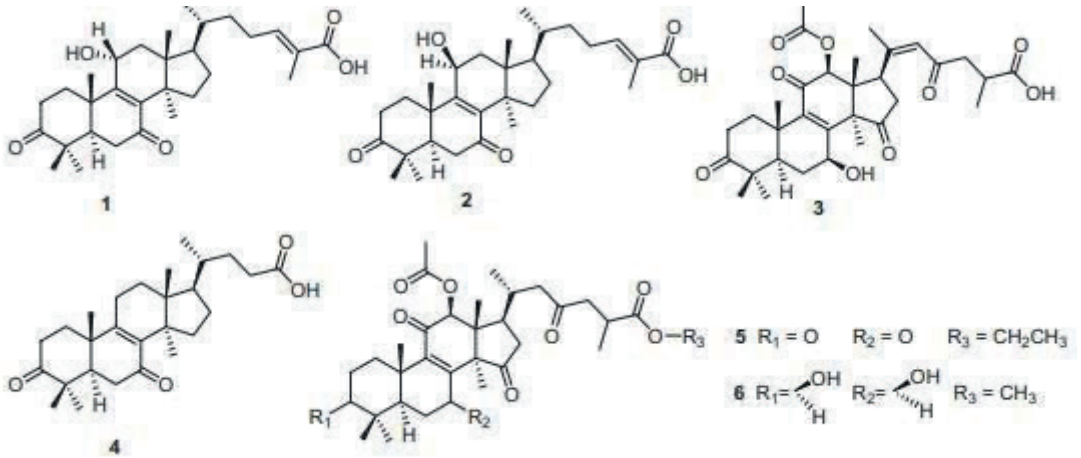


Figure 1: The structure of different triterpenoids [5].

Previous research has proved that many factors, such as solvent concentration, extraction

temperature, time, and liquid/solid ratio, can affect the extraction efficiency from plant materials. Considering all of these factors and their levels, it is a tedious task to optimize extraction conditions, during which not only does the number of experimental runs increase but also the interactive effect cannot be determined [5]. Response surface methodology (RSM) is a statistical method that uses multifactorial modeling to optimize complex processes. It gives a free space wherein the experimental terms can be defined based on the response value, and the levels of factors can be adjusted according to the requirement of the experiment. Therefore, this method may be an ideal strategy for the optimization of triterpenoid extraction from jujube. In addition, the differences in contents of the triterpenes in the materials also affect the composition of the extracts [4]. The compositional profile of bioactive compounds present in jujube is influenced by factors, such as cultivar, geographical environment, processing conditions, and storage conditions. However, because of the difference between the chemical compositions of different cultivars, there are some difficulties in the breeding and planting of jujube varieties, as well as in the quality evaluation and standardization of the developed products [6]. Therefore, it is of great significance for customers and the industry to explore the profiles of triterpenic acids of different jujube cultivars without regional disparity [5].

To optimize the UAE conditions for triterpenoids from jujube fruit using RSM. The effects of extraction temperature, ethanol concentration, time, and the solvent-to-solid ratio on the total triterpenoid yield were studied [4].

To analyze the antioxidant activities and major triterpenic acid profiles in the extracts of different jujube samples (Figure. 2).

To study the differences in the contents of triterpenic acids and antioxidant activities among different cultivars using principal component analysis (PCA) and hierarchical cluster analysis (HCA) . This study provides a comprehensive triterpenoid acid profile of different jujube cultivars, irrespective of the origin differences, and the results provide substantial information on the understanding and utilization of the phytochemical properties of these jujube cultivars for further research [4].

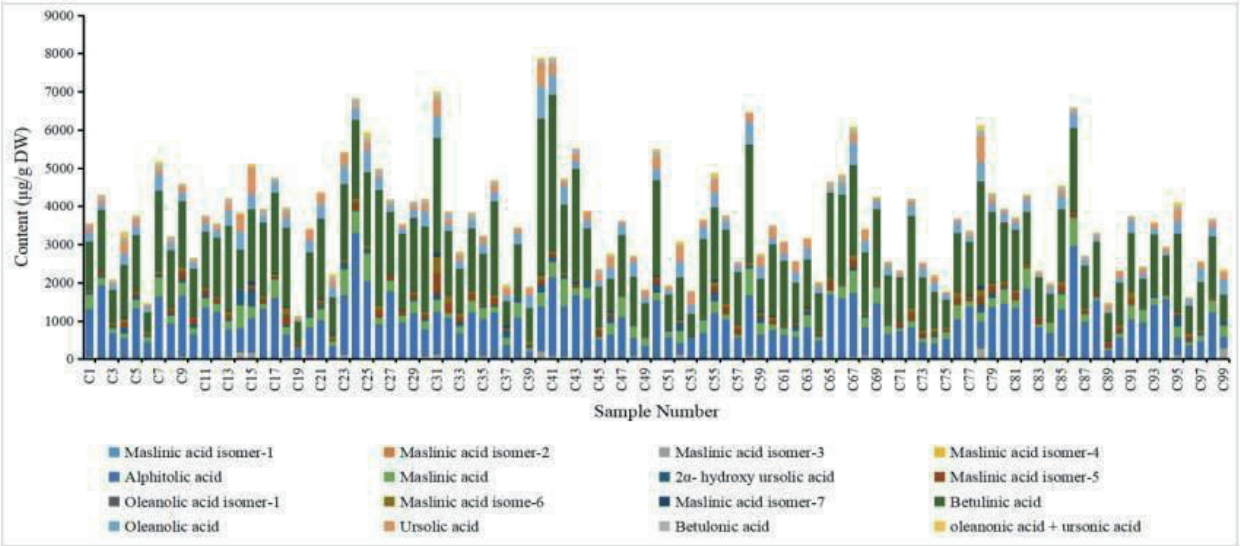


Figure 2: Contents (μg/g dry weight (DW)) of triterpenic acids in different jujube samples[4].

Material and Methods

Plants materials Jujube of 99 cultivars (red maturity stage) were picked from the Germplasm Resources Base of Tarim University at Alaer City of Xinjiang Province, China, by the end of October 2019. Fruits without disease and mechanical injury and uniformly shaped were collected randomly from each side of the trees. After harvesting, all samples were lyophilized and then ground to fine powders and stored below –18 ◦C before analysis [4].

Methods

1- Analysis of Triterpenic Acids by UPLC–MS: The extracts (extracted at optimum conditions) from the 99 cultivars of jujube were analyzed using a Waters ACQUITY UPLC H-CLASS system coupled with a Waters Xevo G2-XS QTof (Waters, Milford, MA). A Waters BEH C18 column (100 × 2.1 mm, 1.7 μm) operated at 30 ◦C was used. Concentrations of the compounds were calculated using the peak areas of the sample and the corresponding standards [7].

2- Soxhlet extraction as the standard technique: Soxhlet extraction was chosen as the standard technique, it is also an HAE. The powdered samples (3.0 g) were extracted with 100 mL of

ethanol by refluxing in a Soxhlet apparatus [8]. To compare the efficiency of the extraction system, the number of cycles in the Soxhlet system was taken into consideration and up to seven cycles were analysed. After the desired number of cycles, the solvent was evaporated under reduced pressure (rotary evaporator Büchi R210, Flawil, Switzerland) to obtain the dried ethanolic extract [9].

3- Determination of the extraction yield: The obtained extraction solutions were filtered through

Whatman paper no. 4 and then evaporated under reduced pressure to remove the solvent [10]. The extraction yield was expressed as a percentage (%, w/w) calculated by dividing the weight of the recovered residue (extract, R) by the weight of the used dry sample (jujube) [4].

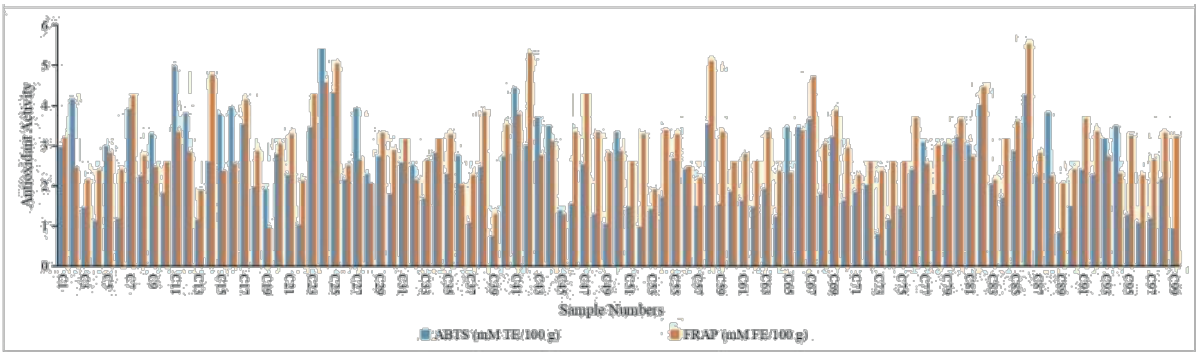
Results and Discussions

Model Fitting*:* The merits of RSM include the use of a lower number of experimental measurements, the provision of a statistical interpretation of the data, and also the identification of the interaction amongst variables. In this study, the Box–Behnken design (BBD) was employed to determine the interactions among X1 (temperature), X2 (ethanol concentration), X3 (time), and X4 (liquid-to-solid ratio), as well as to optimize the UAE conditions [4].

Triterpenic Acid Contents in the 99 Jujube Samples

The triterpenic acids extracted at optimal conditions from 99 cultivars of jujube samples were analyzed by ultra-performance liquid chromatography and mass spectrometry [4].

The major triterpenic acids that widely exist in jujube are one of the main antioxidants with various important physiological and pharmacological properties. Previous researchers have proved that pentacyclic triterpenes, such as maslinic acid, apostolic acid, maslinic acid, oleanolic acid, ursolic acid, glycyrrhetinic acid, betulinic acid, and lupeol, contribute various important physiological and pharmacological properties. For example, ursolic acid and its isomer, oleanolic acid, have been reported to have many beneficial effects, such as antioxidative, antimicrobial, anti-inflammatory, anticancer, antihyperlipidemic, analgesic, hepatoprotective, gastroprotective, anti-ulcer, anti-HIV, cardiovascular, antiatherosclerotic, and immunomodulatory effects [4] (Figure. 3). Betulinic acid has been reported to have anti-



inflammatory, anti -cancer, anti-leukemia, anti-viral, and antihelmintic activities. Due to its selective cytotoxicity against tumor cells and favorable therapeutic index, betulinic acid is considered a promising chemotherapeutic agent against HIV infection and cancers [3]. Maslinic acid has been shown to have antioxidant, anti-inflammatory, antimalarial, and antiprotozoal activities. Therefore, the results of this study provide important guidance for health product development based on jujube [5].

In order to explore the effect of the antioxidant capacity in jujube, correlations among the triterpenic acids and the antioxidant activities were also analysed. As in Table 1, a significant positive correlation was observed between ABTS+ radical scavenging activity and the contents of alphitolic acid, maslinic acid, betulinic acid, ursolic acid, betulonic acid, and total triterpenic acids ($p < 0.05$). Meanwhile, alphitolic acid, maslinic acid, betulinic acid, oleanolic acid, ursolic acid, betulonic acid, and total triterpenic acids also showed a positive correlation with the FRAP value ($p < 0.05$) [4].

The triterpenoid acid profile as of the extracts obtained from 99 cultivars of jujube was further analyzed by UPLC–MS. Betulinic acid, alphitolic acid, maslinic acid, oleanolic acid, ursolic acid, betulonic acid, and 2αhydroxy ursolic acid were found to be the main triterpenoid acids in jujube of different cultivars. According to HCA and PCA, the 99 cultivars were categorized into five clusters, among which cluster 3 had relatively higher contents of most triterpenoid acids [4].

Figure 3: The antioxidant activities of the extracts of different jujube samples. ABTS = 2,2-azinobis (3ethylbenzothiazoline-6-sulphonic acid) FRAP = ferric reducing antioxidant power kit [4].

Table 1: Correlation coefficients (r) of the studied triterpenic acids and the antioxidant activity of jujube cultivars

ABTS	0.1274	0.1943	−0.0398	0.2576 [a]	0.9285 [b]	0.5205 [b]	0.0146	0.1462	−0.0792	−0.0141	−0.051	0.4838 [b]	0.1736	0.3378 [b]	0.464 [b]	−0.009	0.694 [b]	-	0.5205 [b]
FRAP	0.1174	0.0713	0.2629	0.1938	0.5549 [b]	0.9475 [b]	0.1955	−0.1852	0.0911	−0.013	−0.0387	0.3508 [b]	0.6123 [b]	0.2048 [a]	0.0111	0.1888	0.583 [b]	0.4993 [b]	-

Significant at $p < 0.05$. b Significant at $p < 0.01$

The extraction conditions used in the SE technique were selected by applying 7 cycles that correspond to a total extraction time of 4 h. The solvent used was ethanol and the extraction was performed at its boiling point (80 °C). However, depending on the compounds to be extracted, the number of time cycles can influence the recovered quantity. For the studied target compounds (Tr and Ph), there are many scientific references suggesting a significant degradation rate at ethanol's boiling point as the time cycles are prolonged.35 Therefore, to optimize the number of cycles and consequently measure the total content of Tr and Ph at its maximum, the time-cycle (note that each cycle accounts approximately for a 30 min period) of SE for G. lucidum was monitored. The extraction efficiency of HAE and UAE is optimized using RSM.

Conclusions

In this study, the ultrasound-assisted extraction of total triterpenoids from jujube was optimized by RSM. The optimal conditions obtained were as follows: temperature of 55.14 ◦C, the ethanol concentration of 86.57%, time of 34.41 min, and the liquid-to-solid ratio of 39.33 mL/g. The triterpenoid yield was 19.21 ± 0.25 mg/g under the optimal conditions. These results indicate that jujube is a potential natural source of triterpenic acids for the development of functional foods, and the differences in the compositional profile of cultivars may lead to their different applications. UAE is an efficient method to extract triterpenoids from jujube and G. Lucidum and RSM is a useful method to optimize the UAE parameters of triterpenoid compounds from jujube and G. Lucidum. This study would be further contributable to the deep processing and utilization of sample compounds.

Recommendations and Implications

I recommend that the extraction of triterpenoids UAE is a useful method of extraction from organic samples like fruits, vegetables, and furthermore. During examination also seems that HAE and UAE are also helpful for the extraction of triterpenoids from phenolic compounds.

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A Brief Introduction to the Application of Survival Analysis in Medicine

Abubakari Sumaila[1]
Filiz Karaman[2]

Abstract

In Statistics, survival analysis is a popular data analytical technique to analyze time-to-event data. In this technique, the outcome variable of interest to us is the time to the occurrence of an event. For example, the time a patient is diagnosed with cancer to the time of death, the time to wear-out of a newly purchased electronic device, the time to the recurrence of cancer after treatment, the time from marriage to divorce etc. Clearly, this approach of analysis spans different fields of study; from medicine to decision sciences. In this article, the primacy of our work is to review the theoretical basics of analyzing survival data in medicine. We discuss the core components of a typical survival analysis such as estimating the survivor function, hazard function, and fitting Kaplan-Meier curves etc. Further to that, we also look at the application of Cox regression as a semi-parametric modeling approach to fitting survival data. This paper uses Mayor clinic's data on *Primary Biliary Cholangitis*; a condition affecting the liver. The ideas discussed are implemented with the help of R programming Software. Using the survival package, we provide short R-scripts for analyzing the data, as well as checking on the underlying parametric assumptions required.

Keywords: survivor function; censoring; biomarkers; Cox regression; baseline hazard

Discipline: Statistics

Introduction

Survival analysis is a special branch of Statistics used to analyze time-to-event data. What makes this analytical technique special is the fact that we are confronted with data that is neither

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strictly continuous nor strictly categorical, hence, the need for novel approaches to obtain summary statistics, visualizations, methods of statistical inferences, and other forms of modeling. By the term "time-to-event" data, we simply refer to the duration taken for a defined phenomenon to occur (this is known as *event*). For example, the time a patient is diagnosed with cancer to the time of death due to the cancer, the time from a liver transplantation to relapse. Though it is called survival analysis in the medical field, the concept behind it is known by other terms in other fields – in Sociology, it is called *event-history analysis*; in Engineering, it is called *reliability analysis*; in Economics, it is called *duration analysis*.

Historically, the foundation of survival analysis has been built on two approaches; the so-called Kaplan-Meier survival estimator which is the first of the two methods, and was proposed by Kaplan and Meier for estimating survival probabilities [1]. Though the first approach is purely nonparametric, the second approach adopts a semi-parametric approach built around regression analysis. This was introduced by Cox, and is arguably the most used method for analyzing survival data – the so-called *Cox Proportional Hazard Modeling* [2].

There is quite extensive literature on survival analysis where different programming languages are used to for the analyses, notably amongst these are [3], [4], [5]. Sometimes, the discussions around it (e.g. multivariate Cox regression) involve very complex methods that tend to scare some practitioners or make conclusions drawn from the analysis very fuzzy to understand. Furthermore, in Biostatistics or Epidemiology especially, the discussion is often purely theoretical or purely computer programming-laced rather than a combination of both (See [6] and [7] for stata and SAS examples). In this paper, we strive a review approach that complements a theoretical discussion with a practical demonstration in R programming [8]. Undoubtedly, R Software is one of the most widely used programming languages for statistical computation and visualizations. This is partly due to its excellent graphic efficiency, user-friendliness, ever-growing community support, as well as thousands of packages for almost every field of study. Since the focus is on survival analysis in the medical field, we discuss in great extent what functions are needed in analyzing a survival data, how are two survival curves compared, and how far we can go with the interpretation of survival results. In this work, we accomplish that with a practical demonstration with real survival data from Mayor clinic.

The remainder of this work is discussed as follows: We begin a section on motivation for event-

data analysis, and then briefly illustrate few past works on survival analysis, with primary focus on medicine. We follow this up with a section on literature review which is preceded by a discussion on the different types of censoring in survival analysis. Next, a section is dedicated to discussing the survivor function, hazard function etc., the characteristics of these functions. An elaborate discussion on Cox regression is followed. This approach (semiparametric) is particularly compared to a nonparametric estimation approach. To demonstrate what has been discussed, a real data application of survival analysis is done in R Software, and conclude this work a brief concluding remarks.

Motivation

As a time-event data analytical technique, survival analysis has been applied in almost all the widely known disciplines. In such disciplines, the differences in the analysis usually vary only in what is defined as an "event", and how the time horizon for the study is designed. Notably, we cite some of the works done on survival analysis across many fields of study; Suicide [9], Risk of default [10], Divorce [11], Cancer relapse [12], Device reliability [13], Graduation [14] etc. To appreciate the importance of survival analysis in medicine, we arbitrarily cite two examples of past work as motivation, and throw more light on what the objective of those studies were.

Effect of Gender on Colorectal Surgery

With respect to [15], Colorectal cancer (CRC) incidence has been suspected to be less severe in women than it is in men. Following diagnosis of CRC, investigators strove to investigate, retrospectively, the impact of gender on treatment for survivability. The study considered 1796 patients who were considered between November 2004 and December 2017. Survival analysis of this study found a $5-$year hazard ratio for women vs men to be $0.776(Pval = 0.003)$. With respect to tumor localization, the study also found that the survival probability was higher for women, except in a case where patients were older than 80 years old.

Effect of Cancer Type 1 and Type 2 on Renal Cell Carcinoma

In [16], authors focused on finding out if there exist, clinically, significant difference between type 1 and type 2 of papillary renal cell carcinoma (PRCC) in respect of variables such:

demographic factors (*age, ethnicity, gender*, *race*); risk factors (*body mass index, smoking history, neoplasm history, and malignancy history*). One of the findings in this study found smoking to be highly associated with type 2 PRCC survivability.

Brief Introduction to Terminologies In Survival Analysis

In the following, we briefly discuss key concepts of survival analysis — censoring, survivor function, hazard function etc. This discussion is particularly important for later sections on Cox regression, comparisons of survival curves, and diagnostic checks.

Censoring

The time taken for the event to occur is extremely important to the analysis. Nonetheless, this piece of information is sometimes partial. This leads us to the term "censoring". We say censoring has occurred when we encounter a situation where the survival of a subject or participant is not known, usually due to subject(s) being lost to follow-ups, or the event not being observed at the point of termination of the study. Seldom, the event is not observed in all the subjects, and the censored ones may be considered as a form of missing survival data (strictly speaking, this is not the same as NA's). There are different forms of censoring, namely: right censoring, left censoring, and interval censoring. Commonly, right censoring is considered in most case. For example, consider a cancer study where the cancer patients are monitored for a specified period of time. Over the course of time, patients who no longer show up for examination or those who withdraw from the study before it ends can be considered are *right censored* patients. In Figure 1, for demonstration purposes, 4 subjects are shown. Subject *S3* and *S4* both experienced the event (eg. death) within the termination time. Thus, before the study came to an end, both subjects died. However, *S1* dropped out of the study before the study came to an end. In the case of subject *S2*, the participant still had not died at the time the follow-up was concluded. All these are cases of *right censoring*. Below, we summarize the types of censoring you are likely to encounter in survival analysis. For further discussion on this, see [17].

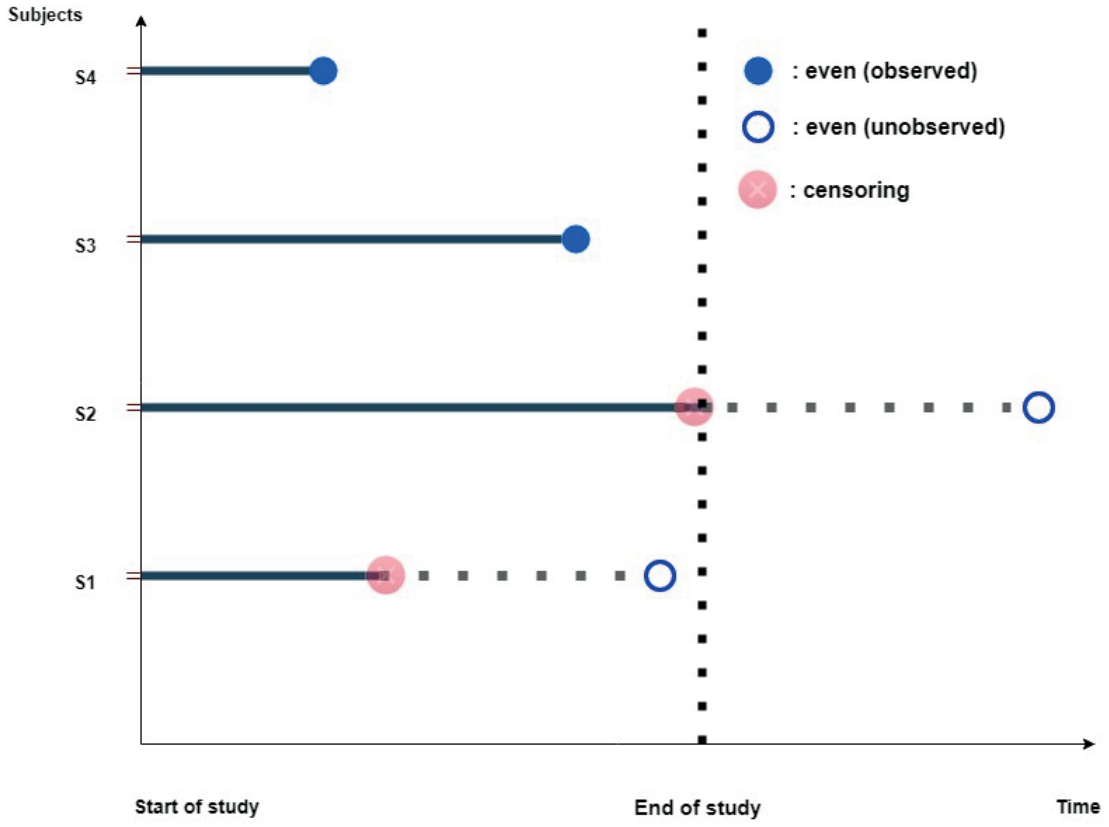

Figure 1: Visualizing right censoring in survival analysis

i. Type I censoring: Here, there is a fixed length of time for the censoring. Subjects begin the study at the same time, and end it at the same time.

ii. Type II censoring: Here, subjects begin the study at the same time, but once a predetermined number is met, the study is ended.

iii. Type III censoring: Here, subjects enter the study at different times, but the length of the study is fixed. E.g. clinical trials.

Survivor Function

We define a survivor function, $S(t)$, as the probability that a participant/subject survives beyond the time t of a random variable T. Mathematically;

$$S(t) = P(T > t) = 1 - F(t) \qquad (1)$$

Intuitively, this refers to the proportion of subjects that have survived beyond time t relative to the total number of subjects (N) in the study. Characteristically, the following properties hold true for the survivor function.

i. $t \in [0, \infty)$

ii. $\forall\ t_1 < t_2,\ S(t_1) < S(t_2)$, i.e $S(t)$ is nondecreasing

iii. $t = 0 \Longrightarrow S(0) = 1$

The probability density function, f, is defined such that;

$$S(t) = \int_t^{\infty} f(\tau) d\tau \qquad (2)$$

which can be rewritten as

$$f(t) = -\frac{dS(t)}{dt} \qquad (3)$$

From Eq. (2), for the random variable, T, the mean life expectation for the subjects/participants considered in the study is obtained as;

$$\mu = \mathbb{E}[T] = \int_t^{\infty} S(t) dt \qquad (4)$$

Alternatively, Eq. (4) can be rewritten as;

$$\mu = \mathbb{E}[T] = \int_t^{\infty} t\, f(t) dt \qquad (5)$$

Another function that is keenly considered in survival analysis is the *hazard function*. It is defined as the instantaneous conditional probability of surviving Δt given that the subject has already survived $T > t$, mathematically expressed as;

$$h(t) = \lim_{\Delta t \to 0} \frac{P(t \leq T < t + \Delta t | T > t)}{\Delta t}, \quad h(t) \geq 0\ \ \forall t \qquad (6)$$

The cumulative hazard function $H(t)$ can be obtained as;

$$H(t) = \int_0^t h(\tau)d\tau \qquad (7)$$

using Eq. (7), we obtain the following;

$$S(t) = exp\left(\int_0^t h(\tau)d\tau\right) = \exp\big(-H(t)\big) \qquad (8)$$

which links together the functions $S(t), h(\tau)$ and $H(t)$.

Nonparametric Estimation (Survivor Function)

As the study progresses and the subjects are being monitored for follow-ups, events occurs in a fashion that may not follow any parametric distribution. Without parametric assumption for the survival times, common nonparametric approaches to estimating survival probability include *Kaplan-Meier* estimation and *Nelson-Aalen* estimation. We briefly cover these two approaches below.

Kaplan-Meier Estimator

For the Kaplan-Meier estimation approach [1], the survival function is defined as;

$$S_{KM}(t) = \prod_{i:t_i<t} \frac{n_i - d_i}{n_i} = \prod_{i:t_i<t} \left(1 - \frac{d_i}{n_i}\right)$$

Recursively written as;

$$S_{KM}(t_k) = \frac{n_{k-1} - d_{k-1}}{n_{k-1}} S_{KM}(t_{k-2})$$

where:

i. n_i: number in the risk set at t_i,

ii. d_i: number of events at t_i

Key assumptions

i. subjects experience the event independently,

ii. censoring of subjects should be *non-informative*. This means that when subjects drop out of the study, it should not be for a known cause that lead to their withdrawal,

iii. prognostic prospect of subjects are not affected by the censored ones.

Nelson-Aalen Estimator

Besides the Kaplan-Meier approach, another nonparametric estimation is the Nelson-Aalen estimator [18]. This method uses an indirect route to estimate the survival function, $S(t)$ by estimating the cumulative hard function. The cumulative estimation is particularly preferred over the point estimation due to the fact that, the cumulative gives a more stable results. The Nelson-Aalen estimator is expressed as;

$$H_{NA}(t) = \sum_{t_i \le t} \frac{d_i}{n_i} = \sum_{t_i \le t} h_{NA}(t) \qquad (9)$$

The survivor function, $S(t)$, is then obtained from Eq. (9) as;

$$S_{NA}(t) = \exp(-H_{NA})(t) = \exp\left(-\sum_{t_i \le t} \frac{d_i}{n_i}\right)$$

Relatively, the Kaplan-Meier estimation is less probably than Nelson-Aalen estimation

Assessing the Significance of Two Survival Curves

So far, we have discussed how to estimate the survivor function and the hazard function. Consider a cancer treatment study where patients are grouped into two separate treatment *arms*, one arm receiving treatment different from the other arm. It would be interesting to investigate which of the two treatment arms gives higher prospect of survivability. We briefly discuss approaches to comparing survival curves.

To compare two survival curves, two approaches are commonly used; the first being *log-rank test* and the second being *Wilcoxon test*. To use the log-rank test, one does not need to make any distributional assumption regarding the survival times. Further to that, subjects in the study are assumed to have equal likelihood of experiencing the event. We refer to this as *noninformativeness* of censoring. Using *R Software*, the `survdiff` function effortlessly accomplishes this task.

Semiparametric Survival Estimation

Cox Proportional Hazard Modeling (CPHM)

Sometimes, the survival of patients depend on more than one variable in the prediction modeling. For example, in oncology, investigating overall survival (OS) of breast cancer patients following a mamography may require investigators to consider the impact of *tumor size, age*, and *cancer stage*. In other words, investigators would wish to know the *joint effect* of biomarkers on the survivability of patients. A regression approach to investigate the survivability of patients taking into consideration one or more covariates is usually achieved through *Cox proportional hazard regression*. This analytic path even allows us to deal with both qualitative and quantitative predictors, whiles at the same time, making it possible for a flexible extension to include more risk factors. In this section, we briefly discuss the Cox Proportional Hazard Modeling (CPHM).

The CPHM is expressed in relation to the hazard function, $h(t)$, such that;

$$h(t,X) = h_0(t) \times \exp\{\beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p\} = h_0(t)\exp\left(\sum_i^p \beta_i X_i\right) \qquad (10)$$

Where

i. $h(t,X)$ is the hazard function on p covariates,

ii. $h_0(t)$ is the *baseline hazard* function,

iii. X_i's is multivariate impact (e.g. covariates like treatment type, gender, race etc.),

iv. $\beta_1, \beta_2, \ldots, \beta_p$ are the regression coefficients,

v. t is the survival time.

Eq. (10) is the generalized expression for p covariates. If the covariates do not have effect on patients' survival, then $X = 0$, and thus reduces the equation to;

$$h(t, X) = h_0(t)$$

It is important to note that the CPHM is *semiparametric* for the simple reason that it does not make any distributional assumption about the *baseline hazard*. However, the model does make the following assumptions for its application (see [19] for details).

i. the covariates have linear effect on the survival time,

ii. the covariates are independent

iii. hazard proportionality (hazard curves do not cross each other)

iv. additivity

Hazard Ratio

Though we have stated that the CPHM does not make any parametric assumption for the $h_0(t)$, there is the possibility that the impact of the covariates does assume parametric form. Thus far, we could rewrite to see the quantile effect of the covariates. In other words, instead of focusing on the regression coefficients, we focus on the *hazard raio (HR)* as shown below;

$$\frac{h(t, X)}{h_0(t)} = \frac{h_0(t)\exp\left(\sum_i^p \beta_i X_i\right)}{h_0(t)} \qquad (11)$$

Taking logarithm to both sides of Eq. (11) gives the hazard ratio as;

$$\log(HR_0) = \log\left(\frac{h(t, X)}{h_0(t)}\right) = \sum_i^p \beta_i X_i \qquad (12)$$

In a simplified fashion, Eq. (12) can be written as;

$$HR_0 = \frac{\text{group hazard}}{\text{baseline hazard}} = \sum_i^p \beta_i X_i$$

This helps us to compare two groups without necessarily worrying about the distribution of the baseline hazard. In the following, we make a little demonstration of this statement. Consider two patients m and m', and assume that they differ in their covariate values. Then, we can form two equation around them as below.

$$h_m(t) = h_0(t)\exp\sum_i^p \beta\, x \qquad (13)$$

$$h_{m'}(t) = h_0(t)\exp\sum_i^p \beta\, x' \qquad (14)$$

The hazard ratio from Eq. (13) and gives;

$$\frac{h_m(t)}{h_{m'}(t)} = \frac{h_0(t)\exp\sum_i^p \beta\, x}{h_0(t)\exp\sum_i^p \beta\, x'} = \exp\sum_i^p \beta\,(x - x') \qquad (15)$$

which is clearly free of t (independent). In summary, the hazard ratio (HR) can be used to assess the *prognostic prospect* of a patient relative to the treatment/control group. HR is either greater than 1, less than 1 or equal to 1 explained below;

i. $HR > 1$: For the treatment group versus control, it means the patients/subjects in the treatment group have increased hazard as compared to those in the control group (i.e. bad prognosis)

ii. $HR < 1$: For the treatment group versus control, it means the patients/subjects in the treatment group have reduced hazard as compared to those in the control group (i.e. good prognosis)

iii. $HR = 1$: For the treatment group versus control, it means both groups have equal hazard (i.e, relative, *no effect*)

Proportional Hazard Assumption Test

Though the CPHM is widely use to investigate the joint effect of covriates on the survival time, its usage is hinged on the validity of the proportionality assumption (PH). It is important to check this, especially when the CPHM is sometimes used to modify estimates of a survival curve (usually termed as *adjusted survival curves*). To test the proportionality assumption, a graphical approach could be used or an approach other than graphical (eg. checking *Shoenfeld residuals* as a Goodness-of-fit test).

Graphically, a simple check involves comparing the expected survival curve with the predicted survival curve, where the observed survival curves are obtained from the stratified estimates of KM curves. This is followed with comparison by a method so-called $\ln(-\ln)$. Another approach is using *Shoenfeld residuals* to check PH assumption. Both approaches are not discussed here (See [19] for further discussion on these methods).

Estimating Cox Regression Coefficients

As we pointed out earlier, measuring the prognostic prospect of patients in the multivariate case lies on estimating the Cox regression coefficients (β's). Owing to the fact that the CPHM is a semiparametric approach, we are unable to find the *maximum likelihood estimation* if the distribution of the baseline hazard is not specified. To overcome this problem, Cox suggested the *partial likelihood* for its estimation. Taking for example a right-censored survival data, the likelihood would comprise two components; one likelihood for patients who observed the event at time t (close of the study), and one likelihood for patients who got censored at the close of the study or by some other decision method for censoring. In this regard, the partial likelihood is expressed as;

$$L(\beta) = \prod_{i=i}^{n} \frac{h_0(t)\exp(\beta^T X_i)}{\sum_{j\in R(t_i)} h_0\,(t)\exp\left(\beta^T X_j\right)'} \qquad (16)$$

where $R(t_i)$ refers to the set containing the subjects at risk at t_i. We seek β that maximizes Eq. (16). Thus;

$$\beta_{ML} = \arg\max_{\beta} L(\beta) \qquad (17)$$

To obtain the coefficients, obtain partial derivatives of Eq. (17) for each covariate such that;

$$\frac{\partial L(\beta)}{\partial \beta_j} = 0$$

It is important to note that it is also possible that, sometimes, there are *ties* in the survival times that need to be dealt with. This calls for a modification to the above equations. However, this modification is not discussed in this article. For elaborate discussion on tied survival times as well as *stratified Cox* regression, see [19].

Real Application: Using R Software

Following the discussion in the preceding sections, we turn our attention to a real application of survival analysis. We do this with *R Software*. Primarily, we use the packages *survival* [20] and *survminer* [21] to accomplish most of the tasks required in the analysis. We use data originally collected at Mayor Clinic on *Primary Biliary Cholangitis (PBC)*, an autoimmune disease that has the potential of destroying small bile ducts in the liver. This data can be accessed easily from the *survival* package in *R*. The data has a total of 418 patients with over *10* covariates such as *age, albumin, ascites, ast, bilirubin, sex, stage* etc. considered in the study. In this analysis, we take a sample of the first 312 patients (See [22] for detailed description).

Kaplan-Meier Curves in Comparison

Here, we fitted the *PBC* data in order to investigate progression-free survival in patients by their gender. For this, we took the first 312 patients and estimated the survival curves by sex (male and female). To assess statistical significance of difference in these two curves, a *logrank test* is used to compute the *pvalue*. As can be seen in the code chunk 1, a$pvalue = 0.038$ was obtained. On the basis of this result, we reject the *null hypothesis* that there is no difference in progression-free survival by sex. Interestingly, we could easily modify the code chunk below to visualize the cumulative hazard. To do that, we add one more *named argument* to the `ggsurvplot` function, i.e, `fun = "cumhaz"`. For demonstration purposes, we arbitrarily choose to sex as a covariate. More covariates could be added for the joint progression-free survival. We will see this later.

```
library("survival")
library("survminer")
library("tidyverse")

#> Warning: package 'tibble' was built under R version 4.2.1

pbc_sampled <- pbc[1:312, ]
fit <- survfit(Surv(time, pbc_sampled$status == 2) ~ sex, data = pbc_sampled)

ggsurvplot(
  fit,
  pval = TRUE,
  conf.int = FALSE,
  risk.table = TRUE, # Add risk table
  risk.table.col = "strata", # Change risk table color by groups
  linetype = "strata", # Change line type by groups
  xlab = "Time in days",      # customize X axis label.
  ggtheme = theme_light(),    # customize plot and risk table with a theme.
  surv.median.line = "hv",    # add the median survival pointer.
  legend.labs = c("Male", "Female"),      # change legend labels.
  palette = c("#E7B800", "#2E9FDF") # custom color palettes.)
```

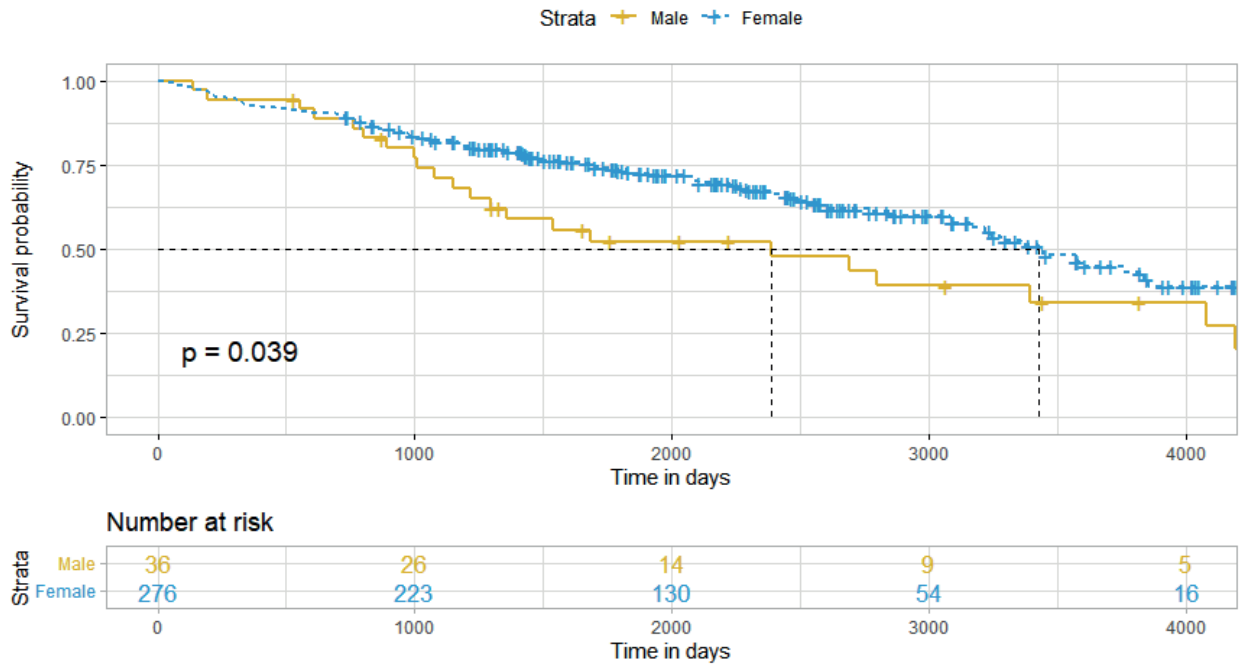

Figure 2: A comparison of progression-free survival by sex (Males vs. Females). Alongside this is censoring event for a 1000-day interval.

Logrank Test

Testing whether there is significance in difference of the two survival curves can be easily in by R by running a *logrank test*. With the survival package, this is achieved with `survdiff` function. The hypothesis considered is:

Hypothesis:
H_0:There is no difference in progression survival between males and females
H_1:There is difference in progression survival between males and females

```
surv_diff <- survdiff(Surv(time, pbc_sampled$status == 2) ~ sex, data = pbc_sampled)
surv_diff

#> Call:
#> survdiff(formula = Surv(time, pbc_sampled$status == 2) ~ sex,
#>     data = pbc_sampled)
#>
#>         N Observed Expected (O-E)^2/E (O-E)^2/V
#> sex=m  36       22     14.6     3.728      4.27
#> sex=f 276      103    110.4     0.494      4.27
#>
#>  Chisq= 4.3  on 1 degrees of freedom, p= 0.04
```

Cox Proportional Hazard Model

As discussed earlier, the Cox proportional hazard model is semiparametric approach to investigating the prognostic prospect of patients given that survival can be explained by the presence of one or more variables. In this case, we demonstrate how it is done using *R Software*. The question we ask here, for example, is: *"How is the hazard of a male or a female increased/decreased is the assessment of progression-free survival of PBC patients?"* To answer this, we run a univariate Cox regression each on the variables believed to have prognostic pointers on progression-free. This is shown in the code below:

```
covariates <- c("age", "sex",  "stage", "bili", "ast", "ascites", "albumin")
univ_formulas <- sapply(covariates,
                        function(x) as.formula(paste('Surv(time,
                                                     pbc_sampled$status == 2)~', x)))
univ_models <- lapply( univ_formulas, function(x){coxph(x, data = pbc_sampled)})
# Results in a data frame format
univ_results <- lapply(univ_models,
                       function(x){
                          x <- summary(x)
                          p.value <- sprintf("%.4f", x$wald["pvalue"])
                          wald.test <- sprintf("%.4f", x$wald["test"])
                          beta <- sprintf("%.4f", x$coef[1]); # coefficient beta
                          HR <- sprintf("%.4f", x$coef[2]); # exp(beta)
                          HR.confint.lower <- sprintf("%.4f", x$conf.int[,"lower .95"])
                          HR.confint.upper <- sprintf("%.4f", x$conf.int[,"upper .95"])
                          HR <- paste0(HR, " (",
                                       HR.confint.lower, "-", HR.confint.upper, ")")
                          res<-c(beta, HR, wald.test, p.value)
                          names(res)<-c("beta", "HR (95% CI for HR)", "wald.test",
                                        "p.value")
                          return(res)
                          #return(exp(cbind(coef(x),confint(x))))
                         })
res <- t(as.data.frame(univ_results, check.names = FALSE))
as.data.frame(res) |>
  knitr::kable(caption = "Univariate Cox regression of PBC data") #, booktab = TRUE)
```

Table 1: Univariate Cox regression of PBC data. A table of regression results: coefficients, confidence interval and pvalues

	beta	HR (95% CI for HR)	wald.test	p.value
age	0.0400	1.0408 (1.0230-1.0589)	20.6000	0.0000
sex	-0.4839	0.6164 (0.3877-0.9798)	4.1900	0.0407
stage	0.8175	2.2649 (1.7768-2.8870)	43.5800	0.0000
bili	0.1489	1.1606 (1.1313-1.1906)	130.9200	0.0000
ast	0.0061	1.0061 (1.0039-1.0083)	29.9600	0.0000
ascites	2.0529	7.7901 (4.8915-12.4065)	74.7500	0.0000
albumin	-1.7957	0.1660 (0.1101-0.2503)	73.5400	0.0000

Table 1 shows the univariate Cox regression of the covariates. For example, the *hazard ratio* for female/male is 0.616. The interpretation given to this is that, hazard associated to the male group is reduced by a factor of 0.616. In other words, the prognostic prospects for the female is relatively better than their male counterparts. The confidence interval for that at 95% is believed to lie between 0.388 and 0.98. As earlier discussed, interpretation for the other covariates take similar line of reasoning. It is important to note that this is only for the univariate case. To run a multivariate Cox regression, the 2 − line code below does the job.

PH Assumption Test

Testing for PH assumption is important in the modeling process. This is because, when the PH assumption is severely violated, inferences from the fitting are most likely going to be misleading. To make a simple PH test in R, we use the function `ggcoxzph()`. In the following, we make a PH test for 'sex' as a covariate. The Shoenfeld residual plot in Figure 3 does not appear to show significant deviation from the solid horizontal line, suggesting no violation of the PH assumption (±2sd).

```
test.ph.sex <- cox.zph(coxph(Surv(time, pbc_sampled$status == 2)~sex,
                             data = pbc_sampled))
ggcoxzph(test.ph.sex)
```

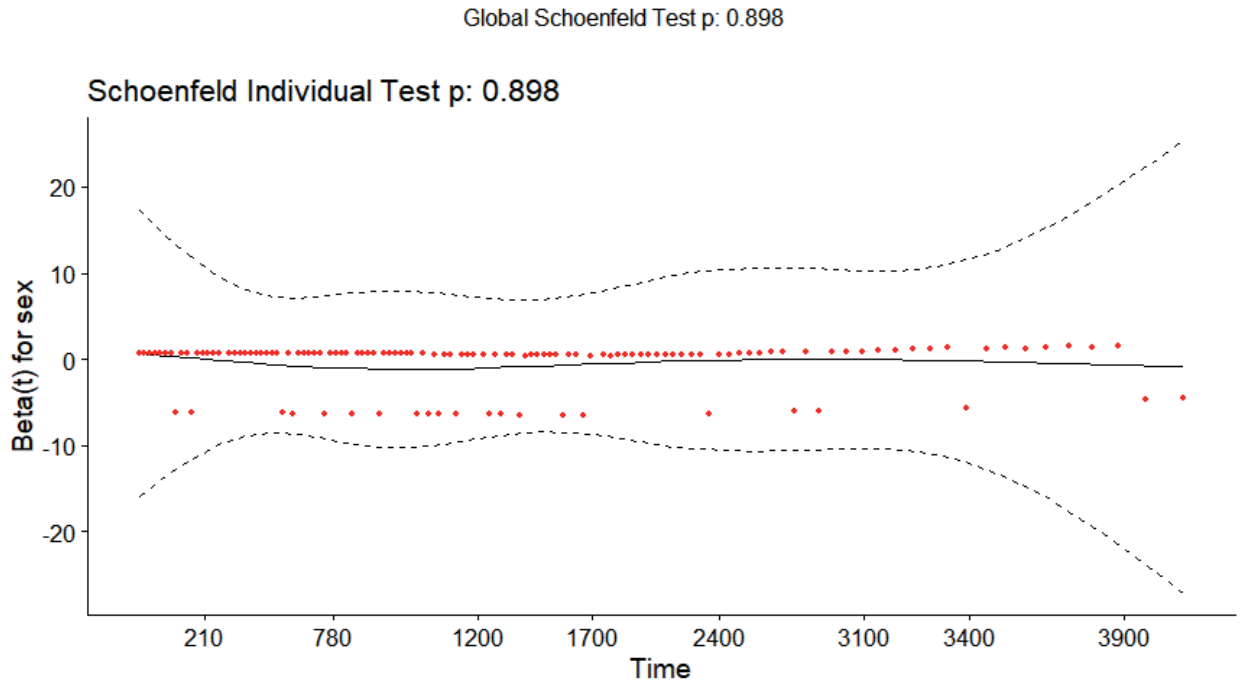

Figure 3: A visualization of the scaled Schoenfeld residuals of sex against the transformed time. Simple approach to testing the PH assumption.

```
test.ph.a <- cox.zph(res.cox)
test.ph.a
ggcoxzph(test.ph.a)
```

Visualization of Hazard Ratios

With the hazard ratio, we are able to draw inferences in regards of the prognostic prospect of the patients in the study. Commonly, a visualization of the *hazard ratios* makes the interpretation easier. In the following, we fit a multivariate Cox regression on the variables: *age, sex, bili, ascites, albumin*, and then follow it up with a visualization of the HRs. This is shown below. From this figure, it is clear *sex* and *albumin* appear to have reduced hazard relative progression-free survival. On the other hand, *bili*, *ascites*, *hepato* appear to have increased hazard on progression-free survival.

```
mult.cox <- coxph(Surv(time, pbc_sampled$status == 2) ~ age + sex + bili +
                    ascites + albumin + ast + hepato, data =  pbc_sampled)
ggforest(mult.cox, data = pbc_sampled)
```

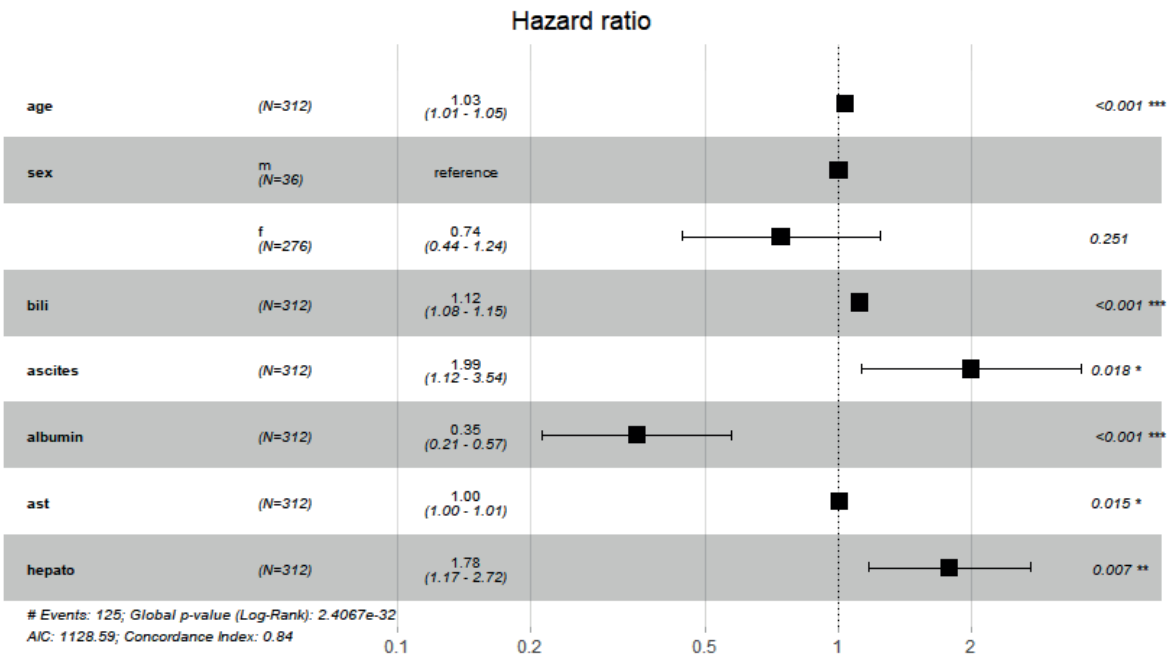

Figure 4: A visualization of hazard ratios in a multivariate Cox regression modeling.

Conclusions

Survival analysis as a modeling technique for time-event data is hugely important in the medical sciences. In this article, an attempt has been made to discus the basics of survival analysis, with a basic demonstration using the PBC data. Usually in medical/biostatistical field, the primary goal might sometimes be related to finding the *Overall Survival (OS)* of patients treated with different forms of medication for a disease, or an attempt at investigating a progression-free survival following a cancer treatment. We have discussed here that, where the covariates do not appear to show serious violation of PH assumption, a Cox regression tends to reveal which biomarkers are highly informative in terms of their prognostic prospect weights towards survivability. However, the nature of covariates we are dealing with can sometimes make the modeling more complicated than the procedures demonstrated in this article. This is especially in a *high dimensional* multivariate survival analysis. Though this work is by no means exhaustive or extensive in discussing survival analysis, we hope what has been described and demonstrated in this article help throw more light on existing literature of survival analysis.

Acknowledgment

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Deposition of CTS Thin Films by PLD and Simulation of Solar Cells Using SCAPS-1D Program

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Serap Yiğit Gezgin[2]
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Abstract

The solar energy is considered as a very powerful alternative for renewable energy generation. CTS (Cu_2SnS_3) is among the new significant materials that are used in thin films based solar cells. Using pulsed laser deposition technique (PLD), CTS thin films were successfully deposited with the mean of home-made target. With its adequate band gap and its big absorption coefficient, CTS is considered as a very suitable absorber material for solar cells. In the current study, we used Solar Cell Capacitance Simulator (SCAPS-1D) program to analyze the output of Mo/CTS/CdS/i-ZnO/AZO thin films based solar cells by introducing the different experimental properties of CTS thin films. Using SCAPDS-1D program, we can calculate separately the influence of the different parameters on the performance of CTS based solar cells. The changing of the temperature and the thickness of CTS thin films have a big influence on the photoelectric properties of CTS solar cell.

Keywords: PLD, Solar cell, simulation, SCAPS, CTS, Thin films.

Discipline: Energy Engineering

Introduction

Because of their low cost of production, great conversion efficiency, and bright future, chalcogenide materials based thin film solar cells are particularly alluring solutions to the

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market's exponential surge in demand for the development and expansion of clean power generation [1, 2]. Due to its suitable characteristics, such as optical band gap (0.9-1.6 eV) [3, 4], p-type conduction properties and an absorption coefficient that exceed>10^4 cm^{-1} [5] CTS (Cu_2SnS_3) material has received a lot of interest recently. Many different deposition methods have been used to grown these thin films such as pulsed laser deposition [6], CBD/electrodeposition [7], sputtering [8] and Co-evaporation [9].

CTS based solar cells were first reported in 1987 by Kuku *et al*. [10]. After that, various deposition techniques and production factors were used to build CTS-based heterojunction solar cells [9, 11-13]. In 2021, Kanai *et al.*[14] used sodium (Na) doped CTS thin films to achieve a noteworthy efficiency of 5.2 %.

Pulsed laser deposition (PLD) [15] is acknowledged as one of the most effective vacuum-based deposition processes for producing thin films with excellent quality employing a variety of deposition parameters (such as energy per pulse, wavelength, substrate-target distance, etc.).

Theoretical study of solar cells has so far been conducted using a number of software tools. Due to its outstanding modeling potentials in solar cell characterization, SCAPS-1D has been the most popular in recent years [6–9] with a various types of solar cells such as: dye solar cells [16], perovskite solar cells [17], thin films based solar cells [18] and organic solar cells [19]. Based on Poisson's and carrier transport equations, SCAPS-1D can calculate the steady-state energy band diagram, interface recombination, and the I-V characteristics of solar cells [10, 11].

In this study, we analyzed the properties of CTS thin films that were deposited using home-made sputtering target for PLD technique. Additionally, this work aims to investigate a heterojunction solar cell using the following device structure: Mo/p-CTS/n-CdS/i-ZnO/AZO. According to the photovoltaic (PV) characteristics of our solar cell, the impact of changing CTS's thickness and working temperature has been researched. Using a highly useful programming tool, SCAPS-1D, our study has provided an understanding of the potential behavior of CTS-based solar cells.

Experimental Process

Deposition of CTS Thin Films

Copper (Cu), Tin (Sn) and Sulfur (S) elemental raw powders of 99.9% purity were mixed by ball milling method with the presence of Ethanol for 6 h. The powder ration were chosen in a stoichiometric way for Cu_2SnS_3 as following: to Cu:Sn:S=2:1:3. Than the resulted solution was dried and pressed into a small sputtering target. The target was subject to annealing process to harden it and to form CTS materials.

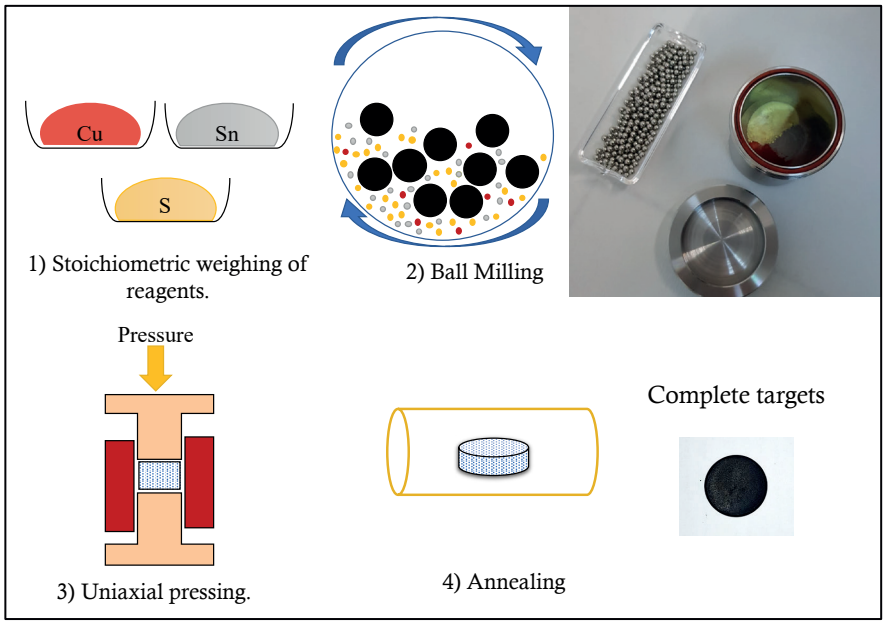

Figure 1: Schematic view of CTS target production process.

CTS thin films was deposited using CTS target using pulsed laser deposition process. The thin film was deposited in an evacuated deposition chamber of a base pressure of 10^{-6} mbar. The energy of laser beam was 15 mJ and the wavelength was 1064 nm with 5 ns pulse width. The deposition duration was 10 min. The resulted film was annealed at a sulfurization temperature of 500°C with the presence of 50 mg of S powder in a vacuumed quarts oven. More details are provided in our previous works [6]. Different analyse were conducted to observe the crystal, optic, composition and morphologic properties of CTS thin films.

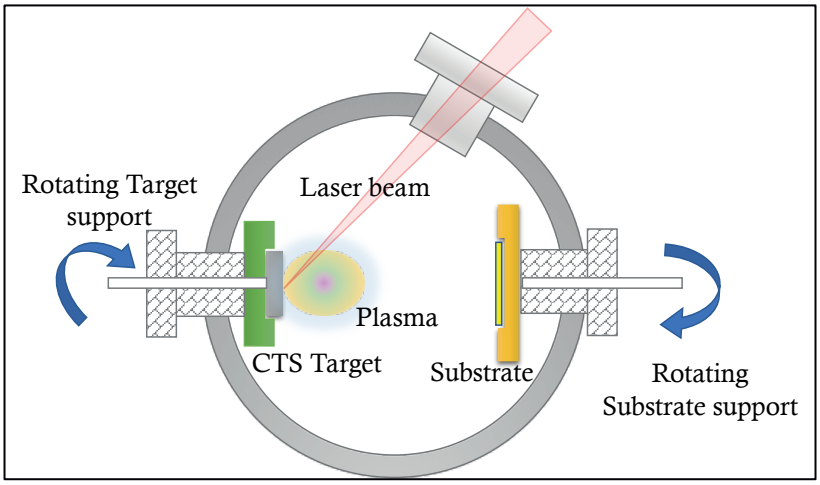

Figure 2: schematic image of PLD system.

Device Simulation and Modelling

Researchers from all over the globe can use the free numerical program SCAP-1D (one-dimensional Solar Cell Capacitance Simulator) to forecast and investigate the behavior of various solar cells under diverse conditions. The ELIS Group developed it at the University of GENT. This application was used to model the Mo/CTS/CdS/i-ZnO/AZO solar cell. Figure 3 displays the design of our construction while Table 1 displays the various parameters for each layer. Some experimental parameters were used in this model such as the bad gap, absorption coefficient and the thickness of CTS thin film.

Table 1. Physical parameters used in this simulation model.

Layers	AZO	i-ZnO	CdS	p-**CTS**
Band Gap (eV)	3.3	3.3	2.4	1.11
Electron affinity (eV)	4.45	4.45	4.2	4.7
Dielectric permittivity (relative)	9	9	10	10
CB effective density of states (cm^{-3})	$2.20*10^{18}$	$2.20*10^{18}$	$2.20*10^{18}$	$2.20*10^{18}$
VB effective density of states (cm^{-3})	$1.80*10^{19}$	$1.80*10^{19}$	$1.80*10^{19}$	$1.80*10^{19}$
Electron/Hole thermal velocity (cm/s)	$1.00*10^{7}$	$1.00*10^{7}$	$1.00*10^{7}$	$1.00*10^{7}$
Electron/Hole mobility (cm^2/Vs)	100/25	100/25	100/25	100/80
Shallow donor density (cm^{-3})	$1.00*10^{19}$	$1.00*10^{15}$	$1.00*10^{17}$	0
Shallow acceptor density (cm^{-3})	0	$1.00*10^{15}$	0	$1.00*10^{16}$
Thickness	200 nm	50 nm	50 nm	Variable

Interfacial defects	
Defect position	CTS/CdS
Capture cross section electrons/hole (cm^2)	$1.00*10^{-15}$
Total defects density (cm^{-2})	$1.00*10^{10}$

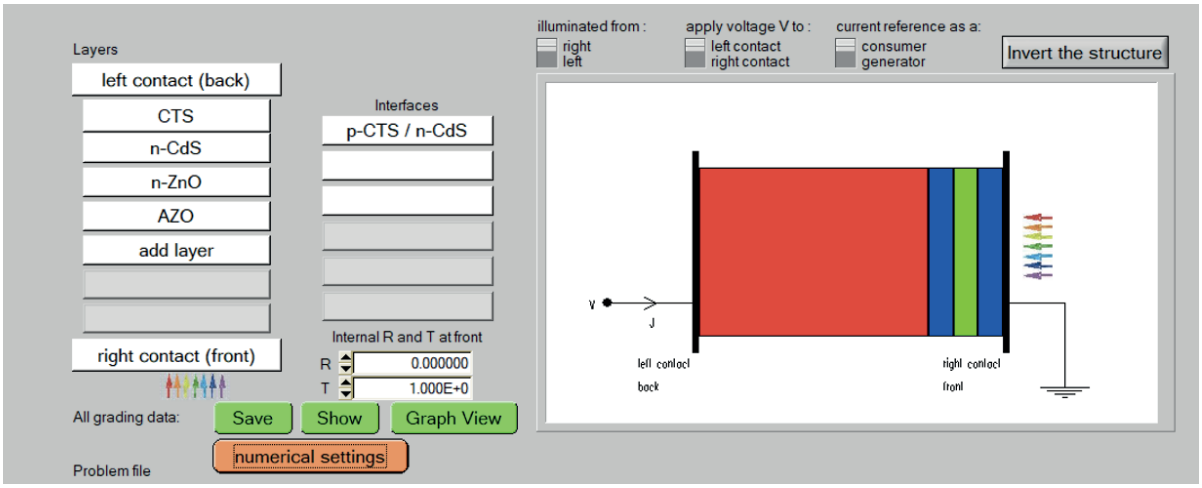

Figure 3: The SCAPS-1D heterojunction solar cell definition panel.

Results and Discussions

CTS Thin Films Properties

Crystal Properties

In Figure 4, we present the XRD pattern of CTS thin film that has been sulfurized at 500°C. The XRD results showed four well defined peaks indicating by that a polycrystalline structure of our films. XRD four peaks are situated at 2θ=28.8°, 33.2°, 47.6° and 56.4° corresponding to (112), (200), (220) and (312) planes, respectively, of CTS tetragonal phase (ICDD:01-089-4714). No visible peaks of secondary phases are visible in the XRD pattern.

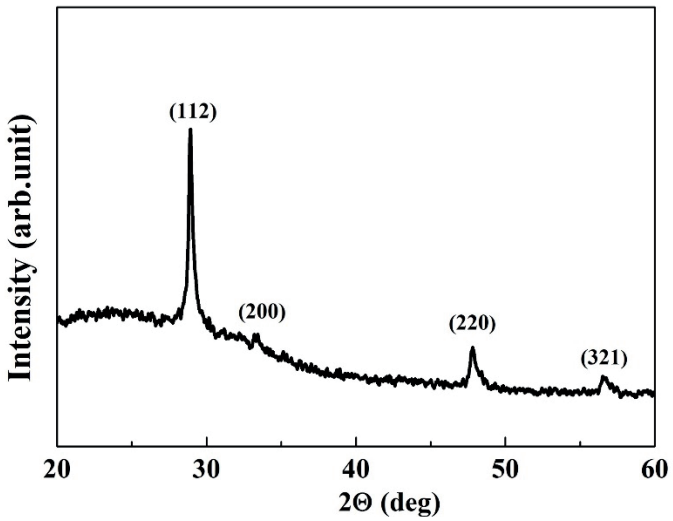

Figure 4: X-Ray diffraction of CTS thin film.

Strain (ε), crystalline size (d) and dislocation density (δ) of CTS thin films are calculated using Eq. 1, Eq. 2 and Eq. 3, respectively, and presented in Table 2 [20, 21]:

$$\varepsilon = \beta cos\theta/4 \quad (1)$$

$$d = 0.94\lambda/\beta cos\theta \quad (2)$$

$$\delta = 1/d^2 \quad (3)$$

θ is the diffraction angle, d is the main size of crystalline, β is the diffraction peak full-width at half-maximum.

The crystalline size calculated from the main peak (112) using Eq. 1 [22] is 25.3 nm which indicate a good crystallinity of the CTS thin films.

Table 2: Thickness, Strain (ε), the crystalline size (d) and dislocation density (δ) of CTS thin film.

Thickness (nm)	**Particle size d (nm)**	**$\delta \times 10^{15}$ (lines/m^2)**	**$\varepsilon \times 10^{-3}$**
458	25.3	1.56	1.37

Optic properties

The absorption coefficient α of CTS thin film is present in Figure 5. The absorption coefficient is calculated from the absorbance spectra using Eq. 4 [23]:

$$\alpha = 2.303(A/T) \quad (4)$$

α is the absorption coefficient, T is the thickness of thin film and A is the absorbance of CTS pure and doped thin films.

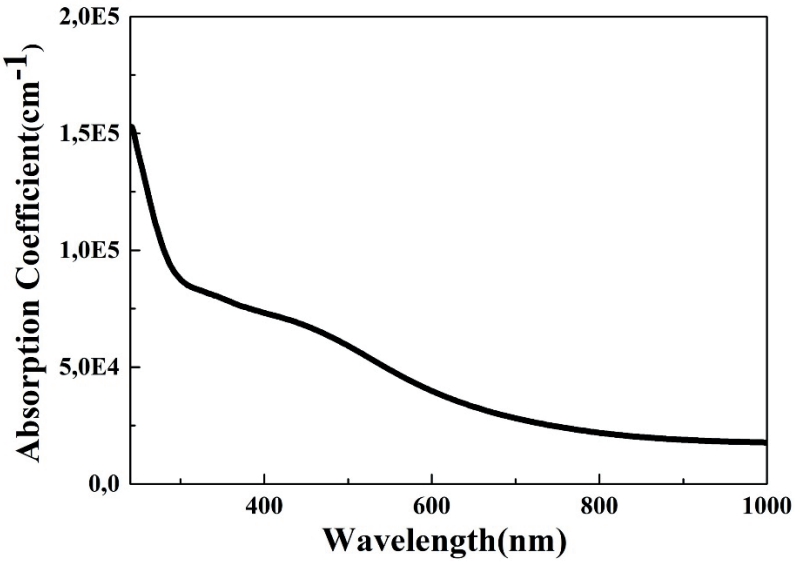

Figure 5: Absorption coefficient of CTS thin film.

α of CTS is $>10^4$ cm^{-1} which make CTS thin films suitable for absorber layer in thin films based solar cells [24]. The optical band gap has a value of 1.11 eV, which corresponded with the values in the literature [25, 26].

Composition and morphologic properties

The top-view surface morphology and the elemental composition of CTS thin films were analyse using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). In Figure 6, we presented the SEM image of CTS thin film. The thin films presented a compact and dense structure with no visible voids or cracks.

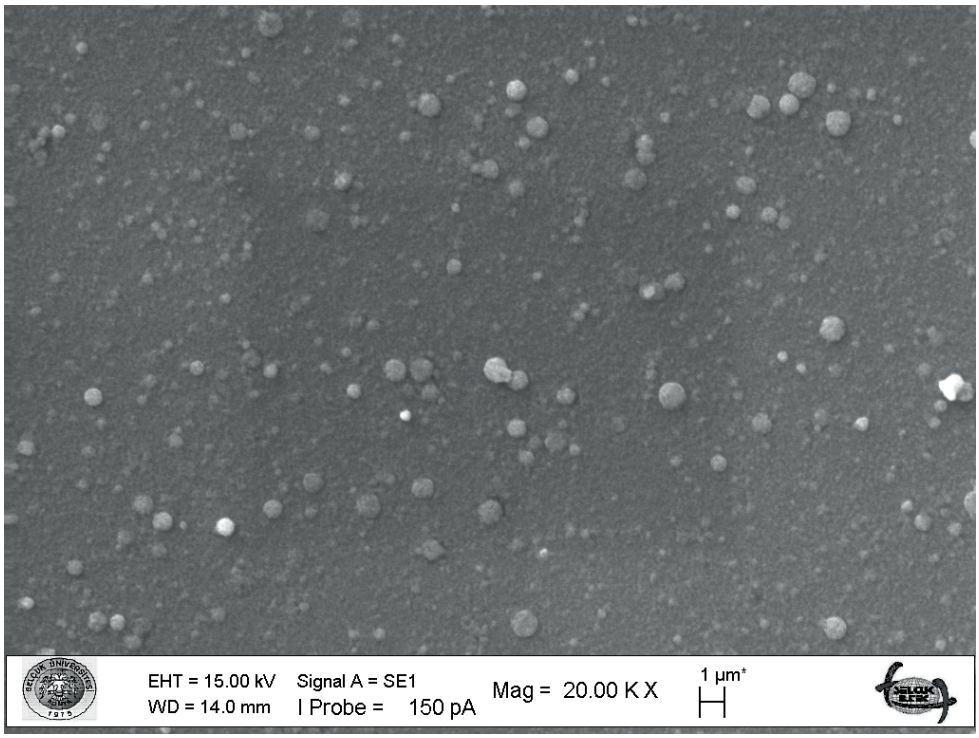

Figure 6: SEM image of CTS thin film.

On the other hand, the atomic compositional concentration and the EDX spectra of CTS thin film are present in Table 3 and Figure 7. We can see the presence of all the CTS material's atoms with near stochiometric ratios.

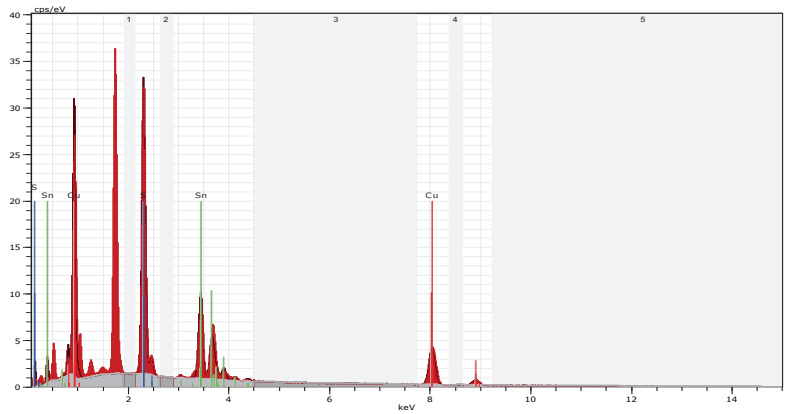

Figure 7: EDX spectra of CTS thin film.

Table 3. The atomic concentration of CTS thin film.

Cu (%)	Sn (%)	S (%)	Cu/Sn	S/Metal
38.95	16.52	44.52	2.36	0.80

Modelling of Mo/CTS/CdS/i-ZnO/AZO solar cell

Effect of CTS thickness

Figure 8 shows the J-V outputs as a result of changing the CTS layer's thickness from 0.3 µm to 1.5 µm. All optoelectrical characteristics of the solar cell attain their maximum values at 0.445 V, 37.39 mA/cm^2, 44.8% and 7.49% for Voc, Jsc, FF, and efficiency, respectively, as the thickness of the CTS thin film increases. The extra absorption of the longer-wavelength photons in the absorber layer is what results in this improvement. So, the quantity of electron-hole pairs produced by photons will increases. Additionally, by increasing the thickness of the CTS thin film, the recombination of the photo-generation electrons in the rear area of the solar cell is reduced [27, 28].

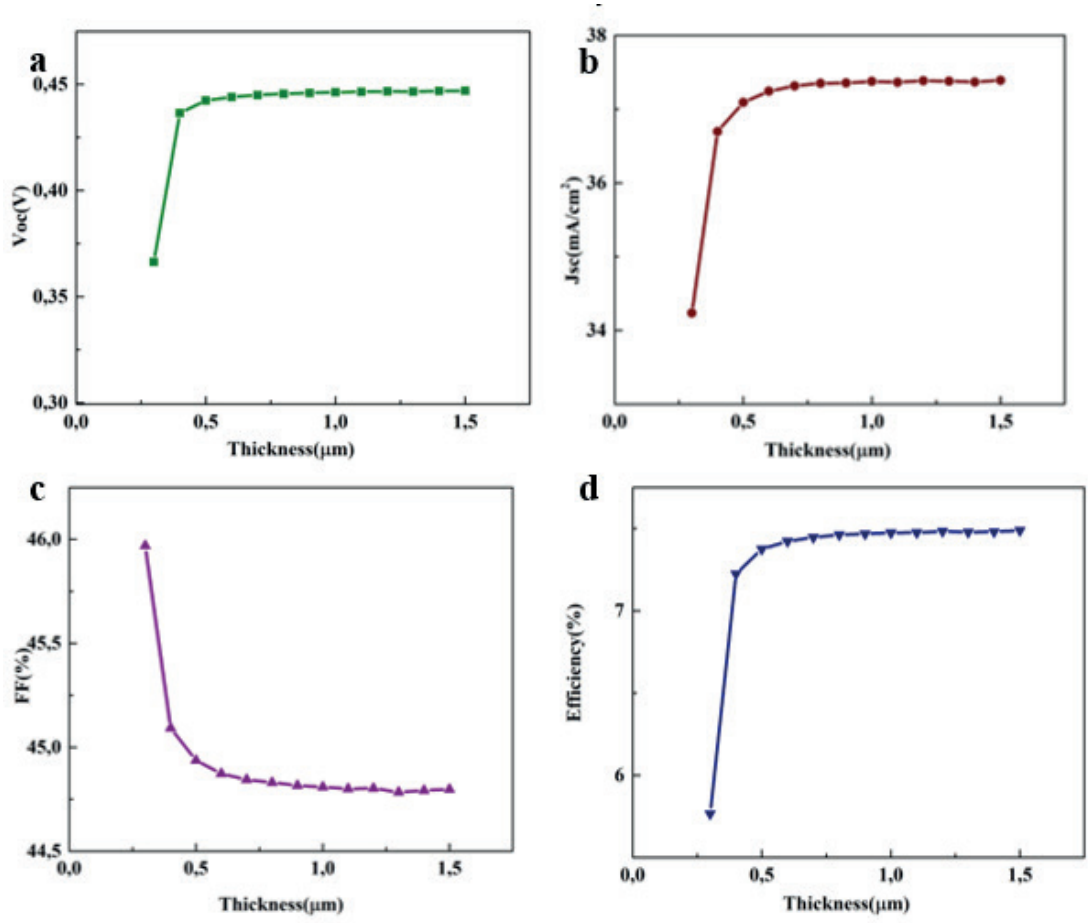

Figure 8: The curves of solar cell photovoltaic performance with variable thickness of CTS absorber layer.

The effect of the working temperature

The temperature of the solar cell cells often varies during the day and throughout the seasons depending on the ambient clime conditions. The change in open-circuit voltage brought on by temperature changes is the main contributor to solar cell temperature dependence. Analysing the fluctuation of current-voltage characteristics according to T is crucial for understanding the prevailing recombination rate [29]. In this work, we investigated the impact of temperature variation on Mo/CTS/CdS/AZO solar cells using temperature variation a range from 260 K to 440 K. Figure 9 shows that the overall impact of temperature on the solar cell characteristics was negative, with noticeable reductions in Voc and FF. J_0 increases appropriately with $\exp\left(-\frac{E_g}{kT}\right)$ [30] as the temperature rises [31]. As a result, we have seen a significant drop in Voc with increasing T [31, 32].

On the other hand, raising the temperature has a beneficial effect on Jsc. The increase in temperature will cause the band gap to narrow [33] and as a consequence, the band-to-band absorption coefficient will rise and the Jsc will improve [29, 34]. When the temperature rises, the increase in Jsc values offsets the decline in Voc, which then causes the efficiency values to fall at T<320. when T>320, Jsc reach a saturation value and the decline of Voc reduces the value of the efficiency to 1.3%

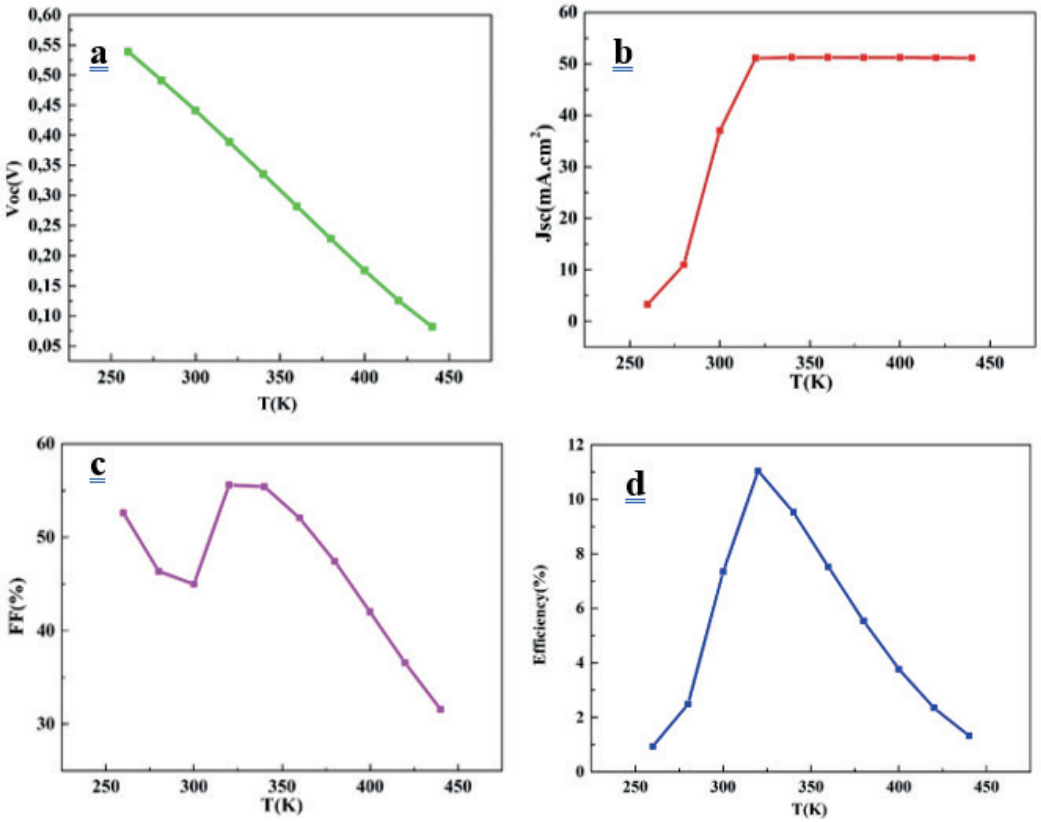

Figure 9: The curves of solar cell photovoltaic performance with variable working temperature.

Conclusions

In this study, we produced CTS thin films using PLD technique. The target material used in this deposition process was home-made from raw elemental powders and then ball milled and pressed then annealed to form a sputtering CTS target. The resulted thin films had a good crystallinity with a tetragonal phase and a 25.3 crystalline size. The optical band gap was calculated from the absorbance to 1.11eV. on the other hand, the SEM and EDX analyses reviled a compact structure with near stoichiometric and copper rich corposant. Using these experimental properties, we introduced the optical band gap, absorption coefficient and the thickness of CTS thin film into SCAPS input data to predict the comportment of Mo/CTS/CdS/AZO solar cells. the effect of CTS thickness was mainly positive while the temperature effect was negative on the overall performance of CTS based solar cells.

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Regeneratıve Agrıculture And Clımate Change: A Case Study Of Morocco

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Abstract

Greenhouse gases have a powerful effect on climate. And since the Industrial Revolution, humans have been adding more of them to the atmosphere, primarily by extracting and burning fossil fuels like coal, oil, and gas, which releases carbon dioxide. These rapid increases in greenhouse gases have caused the climate to warm abruptly. Regenerative agriculture is a way to use the planet's natural systems to rebalance the climate while meaningfully supporting the farmers and ranchers. From rehabilitating entire ecosystems to growing home victory gardens, there are plenty of ways to get involved in regenerative agriculture. Morocco, as a country is characterized by two types of agriculture namely traditional and modern agriculture, and agriculture is the backbone of the economy with the majority of the population relying on agriculture working in farming but climate change is leading to an increase in drought, loss of biodiversity and soil erosion. Knowing how climate change is creating a huge gap between large and small farmers, regenerative agriculture is of paramount importance to them to serve as an alternative to farmers and provide an environmental blueprint. This paper aims to assess the impact of regenerative agriculture in Morocco using descriptive statistics to identify the problems faced by farmers and the solution to the problems. The findings of the study will serve as a gateway for government to implement policies that will benefit farmers and the country at large.

Keywords: Regenerative agriculture, climate change, Morocco, industrial revolution

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Introduction

Today's biggest problems, such as economic growth, climate change, water scarcity, and biodiversity loss, all focus on agriculture. by creating new agricultural models that can renew the earth and feed a population that is expected to reach 9 billion by 2050, we can overcome these difficulties. Although the term "regenerative agriculture" has been around for a while, interest in it has recently increased over the past five years. The canon of "good agricultural practices" includes numerous methods touted as regeneration, including crop residue retention, cover crops, decreased tillage, soil protection, empowering a new generation of farmers, and encouraging animal welfare (Alexandra & Griffin, 2016). Morocco is a geographically varied nation, with agriculture serving as the foundation of the economy and employing more than half of the country's active labor force. Morocco does, however, face some substantial difficulties, particularly with the drought and the destruction of the soil. Moroccan agriculture is distinguished by the coexistence of smallholder traditional agriculture and modern agriculture (DANONE, 2021). Due to improper use of tillage tools, improper handling of crop residues, and improper connections between grain and livestock output, both forms of agriculture are undergoing degradative processes based on research done over the previous three decades.

The Origins of Regenerative Agriculture

Since the late 1970s, the nouns "agriculture" and "farming" have been associated with the adjective "regenerative," but the words "regenerative agriculture" and "regenerative farming" only became widely used in the early 1980s after being adopted by the US-based Rodale Institute. The Rodale Institute has been at the vanguard of the organic agricultural movement for years through its research and publications, which include the periodical Organic Gardening and Farming. Regenerative agriculture, according to Robert Rodale (1983), is "one that, at increasing levels of productivity, expands our land and soil biological production base." It has a high level of inherent biological and economic stability. Beyond the bounds of the farm or field, it has little to no environmental impact (Chelsea, 2018). It creates foods without biocides. The transition to low reliance on non-renewable resources allows for the constructive participation of growing numbers of people. When he produced an "international overview" of regenerative agriculture while serving as director of the Rodale Research Center, agronomist

Richard Harwood gained prominence in the field of global farming systems research (Harwood, 1983). The review makes great efforts to place It also highlights Rodale's claim that Regenerative Agriculture was beyond organic because it included changes in "macrostructure" and "social relevancy" and seeks to increase rather than decrease productive resources. Harwood (1983) summarizes the "Regenerative Agriculture Philosophy" in ten guidelines. This philosophy, he continues, emphasizes three things:

1) The interdependence of all farming system components, including the farmer and his family;

2) The importance of the system's numerous biological balances; and

3) The need to maximize desired biological relationships and minimize the use of tools and methods that sabotage those relationships.

Box 1. Points summarizing the Regenerative Agriculture Philosophy as presented by Harwood (1983: 31)

1. Agriculture should provide high quantities of nutrient-dense, biocide-free food.

2. Agriculture should improve the depth, fertility, and physical properties of the higher soil layers to increase rather than diminish soil productivity.

3. Better crop nutrition is ensured by nutrient-flow systems that fully incorporate soil flora and fauna into the pattern. These systems are more effective and less harmful to the environment. By creating a fresh upward flow of nutrients in the soil profile, these systems lessen or eliminate negative environmental effects. Such a procedure is a soil genesis procedure by definition.

4. In order to stabilize crop output, biological interactions should be used instead of synthetic biocides.

5. Chemicals that interfere with how the farming system is biologically structured (like today's synthetic fertilizers) should not be used.

6. Regenerative agriculture necessitates a close bond between the system's participants and manager due to the biological structure of the system.

7. It is best to use integrated systems that are mostly nitrogen self-sufficient due to biological

nitrogen fixation.

8. In order to prevent the use of hormones and the prophylactic use of antibiotics, which are later found in human food, animals in agriculture should be fed and housed in a certain way.

9. More jobs should be created as a result of increasing agricultural productivity.

10. To shut nutrient-flow loops, regenerative agriculture involves planning at the national level, but it also relies heavily on local and regional autonomy.

Francis et al. (1986) link regenerative agriculture closely to organic and "minimal external input agriculture" in what is likely the first published publication on the subject. They also emphasize the significance of biological structuring, progressive biological sequencing, and integrated farm structuring. They also link it to several "particular technologies and systems," such as nutrient cycling, crop rotation, integrated pest management (IPM), nitrogen fixation, and "weed cycling."

Regenerative agriculture experienced an initial surge of enthusiasm before disappearing for over two decades before picking up steam again. We examine the degree of adoption of the terms "regenerative agriculture" and "regenerative farming" in the academic and public arenas to serve as an example of this. The use of these phrases in novels first peaked in the mid-late 1980s, as shown in Figure 1, but by the mid-2000s they had all but vanished. After 2015, the prevalence of regenerative agriculture then sharply rose. It is crucial to note that Regenerative Agriculture has been mentioned in publications significantly less frequently between 1972 and 2018 than phrases like Sustainable Agriculture, Organic Agriculture, Organic Farming, and Agroecology.

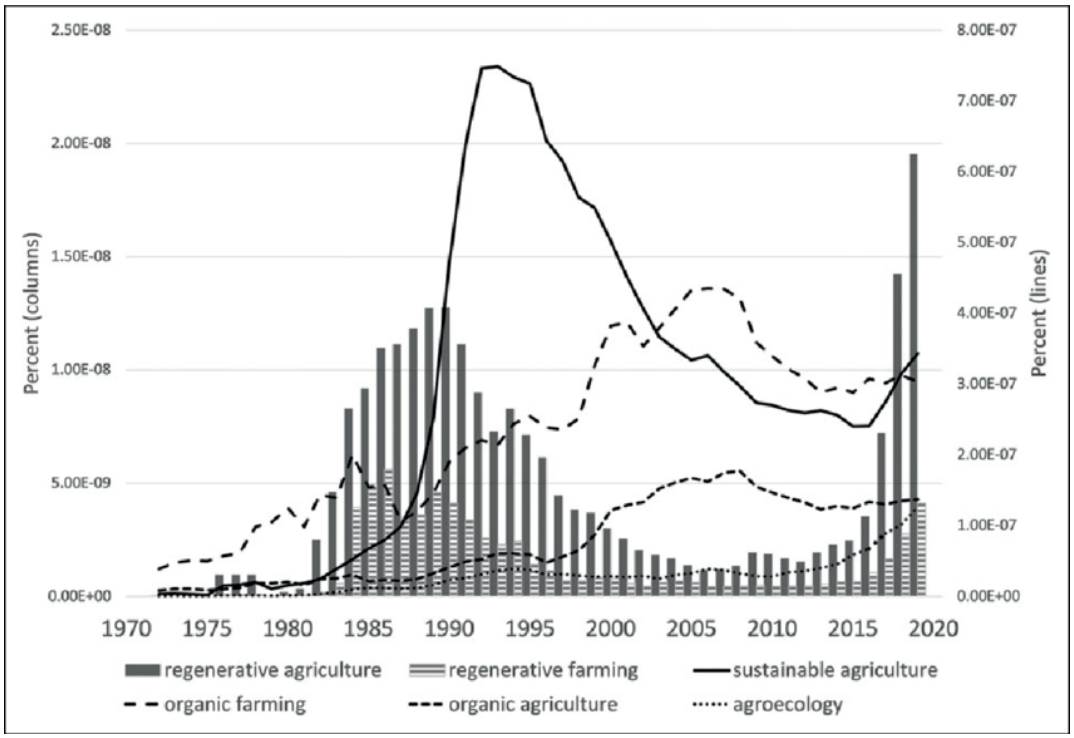

Figure 1: The frequency of key terms in books (3-year rolling averages)

Source: Woody, H. (2020)

Regenerative Agriculture and Regenerative Farming first appear in news reports referencing the Rodale Institute in 1983 and 1986, respectively (Figure 2a), and neither phrase was used in more than 15 news stories annually until 2009. After 2016, their combined usage skyrocketed, and each year after then, their frequency doubled, reaching 6163 news articles in 2020. To put this into perspective, in 2020 there were 6,870 and 18,301 news stories about organic farming and agriculture, respectively. In the more scholarly literature, only seven more studies are found by Web of Science in the first 30 years after Francis et al. (1986) with the terms "Regenerative Agriculture" or "Regenerative Farming" in their title or abstract (Figure 2b). The year 2016 signaled a turning point in academic interest, and by the year 2020, 52 scholarly publications had been published, with a total of 250 citations. Thus, even though the terms "regenerative agriculture" and "regenerative farming" have been in use since the early 1980s, they have not yet gained as much traction as terms like "sustainable agriculture" or "organic agriculture," which are also related. Since 2016, they have become much more common in books, news articles, and online content, which is a reflection of the fact that many NGOs (such as The Nature Conservancy, the World Wildlife Fund, Greenpeace, and Friends of the Earth),

multinational corporations (such as DANONE, General Mills, Kellogg's, Patagonia, and the World Council for Sustainable Business Development), and charitable foundations have adopted them (e.g. IKEA Foundation).

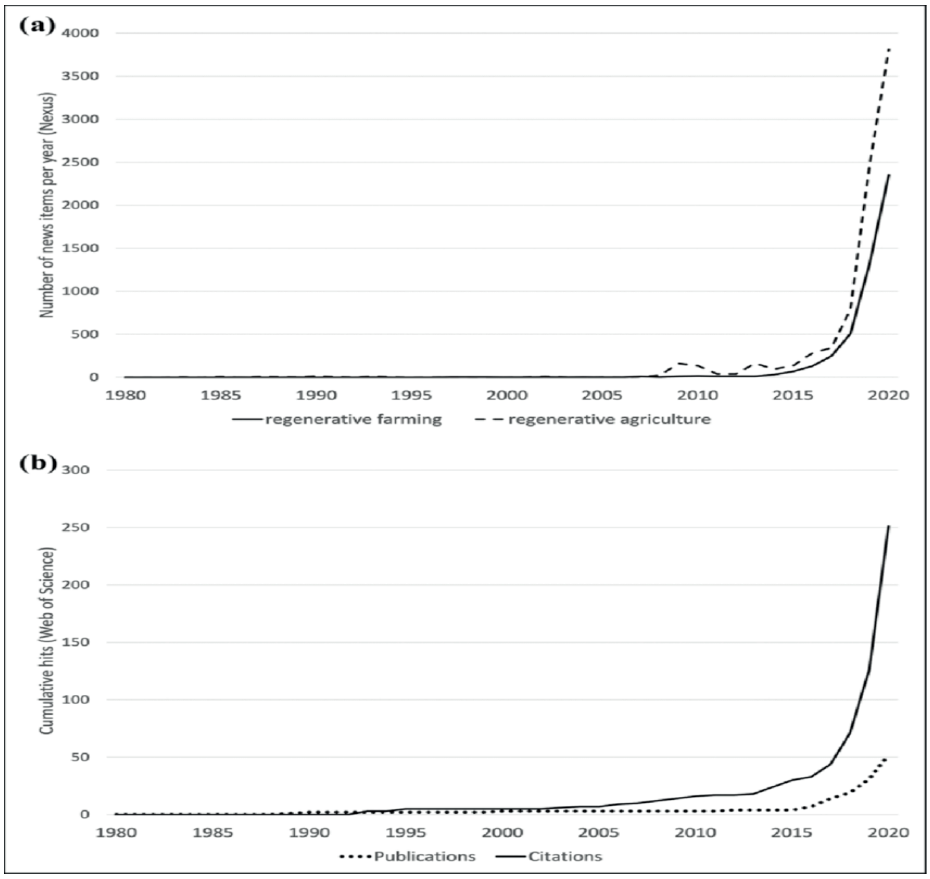

Figure 2: (a) Occurrence of Regenerative Agriculture or Regenerative Farming in news items and (b) Academic peer-reviewed publications on Regenerative Agriculture or Regenerative Farming.

Source: Woody, H. (2020)

According to the statement that "Consensus is mounting for a single, standardized definition for food grown in a regenerative manner that restores and maintains natural systems, like water and carbon cycles, to enable land to continue producing food in a manner that is healthier for people and the long-term health of the planet and its climate," regenerative agriculture should be defined around the outcome. Regenerative Organic Certified is a particular certification program that was launched in 2017 in the USA under the direction of the Regenerative Organic Alliance, an organization in which the Rodale Institute plays a significant role. The three pillars of Soil Health, Animal Welfare, and Social Fairness serve as the foundation for certification;

it is believed that each of these pillars can be confirmed using current certification standards. The development of this new standard was motivated by a perceived need to go beyond the requirements of the USDA Organic Certification program. Improvements to soil health, the wider ecosystem, human health, and economic success are the most frequently appearing topics connected with regenerative agriculture, according to an examination of peer-reviewed studies (Schreefel et al., 2020). Regenerative agriculture, according to the authors, is "an approach to farming that uses soil conservation as the entry point to regenerate and contribute to multiple provisioning, regulating, and supporting ecosystem services, to improve not only the environment but also the social and economic dimensions of sustainable food production." While Regenerative Agriculture is categorically a form of organic agriculture according to some groups, others are willing to use agrochemicals sparingly. However, from an agronomic standpoint, the two difficulties associated with regenerative agriculture most usually are:

1. Restoring soil health, which includes carbon (C) capture to lessen climate change

2. Restoration of biodiversity.

Additionally, regenerative agriculture is essential for addressing consumer and community concerns, which are increasingly focused on the environment. A Boston Consulting Group survey of more than 3,000 respondents in eight countries found that 75% of participants believe environmental concerns are just as important as health concerns, and 70% of participants are more aware now than they were before the covid-19 pandemic that human activity threatens the environment. It is hardly surprising that consumers are altering their behavior given that global searches for sustainable goods have increased by 71 percent since 2016 and that businesses marketing sustainable goods are expanding seven times faster than those marketing conventional goods.

Source FAO: 1/3 of the earth's surface

70% of freshwater consumption is according to the World Bank

1/5 of global GHG emissions come from IPCC

33 percent of soils have moderate to severe degrading conditions Source Global Land Outlook of the UN

One million plant and animal species are in danger of being extinct, and the main causes are land use change and agriculture. IPBES as a source

Dr. Rattan Lal, a soil scientist and the recipient of the 2020 World Food Prize, claims that increasing the carbon content of the planet's soil by merely 2% will reduce greenhouse gas concentrations in the atmosphere to safe levels.

Soils that are treated organically can hold up to 40% more water than those that are managed conventionally.

In some regions, innovative regenerative farming has caused water springs that dried up several years ago to start flowing again;

Soil: depth of tillage, percentage of soil covered, number of species, depth of crop rotation, monitoring, and organic matter content.

Biodiversity includes methods for controlling weeds and pests that reduce the use of chemicals, the percentage of natural habitats on agricultural fields, and the percentage of locally sourced animal feed.

Water: using natural resources, incorporating buffer zones, and managing irrigation. 2 billion hectares of land are degraded worldwide. We must immediately switch to a more sustainable agriculture model that will increase soil health, aid in predicting future climate shocks, feed an expanding population, pay farmers a reasonable living wage, and lessen our reliance on fossil fuels. Director of Conservation Programs at WWF France, Arnaud Gauffier

Our water stewardship and regenerative agriculture programs place a significant emphasis on the health and sustainability of our agricultural supply chain because agriculture accounts for 89 percent of our total water footprint.

Regenerative Agriculture Practices, The Soil Crisis, Biodiversity Crisis, And Climate Change

The constraints of the conventional farming model have been shown. The rate of climate change is rising. The diversity of life is dwindling. As water becomes more scarce, so does the quality of the water. Farmer poverty is still pervasive. If things continue as they are, it will take

us 50 years to locate enough topsoil for agriculture to feed 9 billion people (Mrabet, Fadlaoui, Eric, 2012). We must adopt a new paradigm that will turn agriculture from a problem to a solution and bring us all closer to nature (Oliver, 2020).

Resources can deteriorate through agriculture, but they can also be renewed. When it comes to removing more carbon from the atmosphere, soaking up extra rainwater, and promoting biodiversity, soils can be a powerful force (Mrabet, Fadlaoui, Eric, 2012). Regenerative agriculture is becoming more and more important for health since there is mounting evidence that the quality of the soil affects the quality of food.

The security of mankind on Earth is at risk due to climate change caused by human activity, making this the most important time in the history of our species. Around 52 GtCO 2e worth of greenhouse gases were emitted annually worldwide in 2012. If we are to have a realistic chance of keeping global warming to 1.5°C, above which we dare not pass, these emissions must shortly decline to a net of 41 GtCO 2e (Mrabet, Fadlaoui, Eric, 2012).

A zero-carbon economy devoid of fossil fuels is necessary to solve the long-term climate equation. It is commonly understood that the transition to a new low-carbon economy won't happen anytime soon since the necessary technology, markets, political systems, and social structures aren't developing quickly enough.

1) An unacceptably high amount of warming will be locked in throughout the decades it will take to decarbonize the economy.

2) As each year of inaction goes by, it gets harder and harder to hold onto hope for the future of our planet. Our trajectory is one of too little, too late.

Simply put, recent statistics from farming systems and pasture trials around the world demonstrate that switching to widely accessible and reasonably priced organic management practices—what we refer to as "regenerative organic agriculture"—could capture more than 100% of the present yearly CO2 emissions. By maximizing carbon fixation and limiting carbon loss after it is returned to the soil, these techniques aim to reverse the greenhouse effect.

Morocco

Moroccan agriculture is divided between smallholdings that produce food primarily for local markets and farmers' livelihood and a modern industrial sector that produces food largely for export (Megan, 2015).

Although more than 70% of farmers operate on less than five hectares, this only makes up 25% of the total area under cultivation because large farms predominate in fertile areas. Naturally, the large farms make significantly more money than the typical family farm, almost nine times as much. Numerous small farms struggle with issues that limit their ability to raise revenue, such as hazy land ownership, a lack of infrastructure or credit, and inadequate technical and marketing support (Sarah, Maggie, Shahnaz, Shane, 2018). Government programs are inaccessible to small farmers without registered land, and even then, many favors larger farms.

Morocco's diversified geography leads to a wide range of agricultural products, from cereals and vegetables to fruits and nuts. Major exports from the nation include olives, argan oil, citrus, and almonds. Fish exports account for 55% of all food exports, making them another big business. As one of Morocco's greatest assets, this diversity should be praised for making a considerable contribution to the nation's agricultural sustainability and food security (Mrabet, Fadlaoui, Eric, 2012).

More than 80% of the rural population in Morocco depends on the revenue from livestock, which is another important area of Moroccan agriculture. Animals provide farmers with a financial cushion and protection from the effects of drought. 53 million hectares of vast rangelands are home to large herds of cattle, sheep, goats, and dromedaries (Arabian camels) (Rachid, 2014).

Drought and Soil Erosion

The effects of climate change and the growing occurrence of drought are significant issues for Morocco. Morocco has experienced droughts every three years for the previous few decades. By 2050, temperatures are expected to increase by three degrees while precipitation is expected to decrease by 10%. The expected rise in water consumption over this time frame is six times higher (Alexandra & Griffin, 2016). This has significant ramifications because the majority of cropland is situated in regions with yearly rainfall totals less than 400mm. The government is

attempting to desalinate seawater for use in agriculture, despite the enormous task it has in reducing the effects of climate change (Mrabet, Fadlaoui, & Eric, 2012).

With soil erosion affecting over half of the area and desertification threatening 80% of it, these protracted droughts are worsening soil deterioration. A growing population is putting increasing strain on resources and removing natural vegetation as more area is turned over to farming (DANONE, 2021). For instance, the Rif Mountains have one of the worst erosion rates in the entire world. Conflict is being caused in areas where land is jointly owned and used for grazing by this issue, which is also contributing to water pollution by increasing the levels of siltation in reservoirs and oceans (Chelsea, 2018).

Major Soil Constraints Affecting Sustainable Agriculture Production And Development

The most significant process of soil degradation in Morocco is soil erosion. 100 million tons of soil are estimated to be lost year overall.

Ground depth (limits soil depth). This is a significant obstacle that Morocco's agriculturally fertile land must overcome. According to a survey conducted across the nation, over 2 million acres might be reclaimed by subsidence and stone removal.

The biggest danger to the nation's food security and output is erosion. Under a rainfall simulation, the conventional tillage approach based on offset disking resulted in runoff and soil loss and encouraged surface sealing (Dimanche and Hoogmoed, 2002). As demonstrated by Moussadek et al. (2011a) in the Zaers region, the soil cover is the most significant component that influences the rate of water infiltration into the soil, hence lowering runoff and erosion (Fig. 3). Moussadek et al. (2011a) compared water runoff and soil loss in a conventional tillage system and NT systems with crop residues removed (NT0) and with 50% of crop residues on a Vertisol using a rainy simulator.

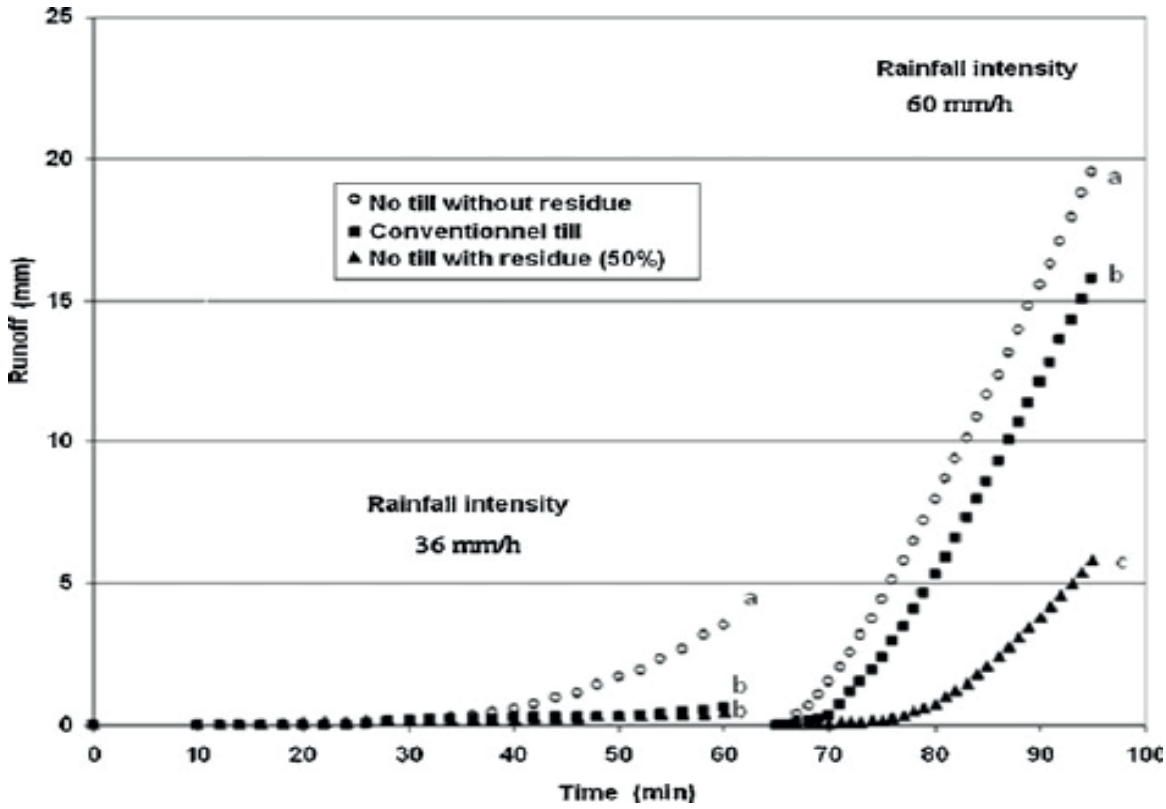

Figure 3: Runoff loss as affected by tillage and NT residue removal under two rainfall intensities in Zaers region

Source: Moussadek (2011a)

The NT system promotes rainfall infiltration, which increases the quantity of soil water available to plants in heavy textured soils (Bouzza, 1990), and residue mulch prevents crust development in structurally weak soils. As a result, the NT system improves the infiltration properties of the majority of soils. Better aggregate stability and bioporosity under NT are the cause of these elevated infiltration rates. Water infiltration rates under well-managed NT are higher over long periods than under CT systems, as demonstrated for a Vertic Mollisol in the Chaouia region (Fig. 4). These findings concur with those made for the Sais region on clay soil by Dimanche (1997).

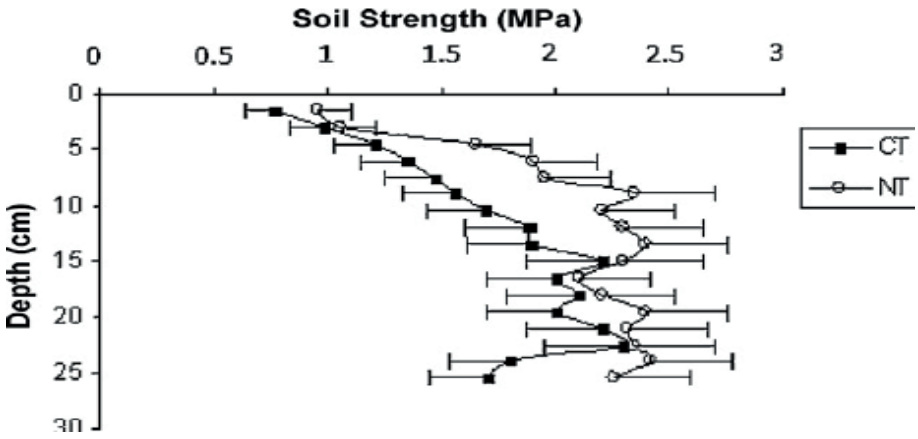

Figure 4: Impact of tillage system on infiltration process in Sidi El Aydi clay soil. Conventional tillage -offset disking

Source: Mrabet (2008).

By expanding irrigation infrastructure and promoting drought-resistant crops, the government is striving to address these challenges. But contemporary methods like high tillage and intense agriculture under irrigation are causing soil organic matter to disappear (Rachid, 2014). Perhaps using more conventional methods can help in some cases. The use of livestock manure to increase soil fertility is extremely valuable, and mixed farming appears to be a significant element of Moroccan agriculture (DANONE, 2021).

Agriculture sustainability is becoming more and more crucial for nations throughout the world due to growing populations and food shortages. One such nation where the success of the agricultural industry is highly stressed by its arid climate is Morocco. Morocco must continue to adapt in the agricultural sector due to its mountains, desert, rainy season, which is marked by floods and mudslides, and dry seasons, during which the soil erodes and crops struggle to survive. The agriculture industry in Morocco contributes 15% of the country's GDP (International Trade Administration, 2020). The growing consequences of climate change pose a persistent danger to this crucial sector of Morocco's economy. More than ever, the nation must concentrate on sustainable methods to safeguard the future of the agriculture sector (International Trade Administration, 2020).

The Green Morocco Plan

The Green Morocco Plan, which started in 2008, is the government's attempt to address its

agricultural issues. The two cornerstones of this scheme are the huge intensive farms and the small subsistence farms, respectively. It seeks to boost productivity, double the value of agricultural output, and enhance food security. It claims to maintain the "social character" of Moroccan agriculture while moving in the direction of deregulation and modernization (Jihane, 2018). A team of 1,500 programmers will be needed to complete the idea, which will cost more than $10 billion. It includes building dams, increasing irrigation, and switching to crops that are more suited to the climate. Planting fruit and olive trees on formerly used grain fields is one of its components (Alexandra, & Griffin, 2016).

Even while many of these programmings are useful, some people feel that the Plan favors large-scale, intensive farming. There are worries that the government is promoting an intensive agriculture model that depends on the free market and the global market, with the help of commercial seed and chemical companies and international development groups (DANONE, 2021).

Co-Operatives, Organic Farming, and Community-Supported Agriculture

Community-supported agriculture and cooperatives are two ways to assist small farmers (CSA). Cooperatives can offer training and support, as well as split the cost of equipment and packaging. Trees are being sold at a fraction of the price of huge commercial nurseries by community-managed tree nurseries. Small farmers can do better in the global economy by cooperating (Ken, Renske, Jens, & James, 2021). By pooling their produce, they can compete with bigger farms.

The desire to increase sustainability among farmers is widespread. Farmers are testing out more sustainable agricultural methods at a field school run by a cooperative in a community not far from Taounate. Farmers are now able to conduct their studies and come up with their solutions thanks to this.

In Morocco, organic farming is also gaining popularity. Sala Almoustaqbal, a CSA organization, started manufacturing organic food that it sells directly to consumers. With more than 100 families on the waiting list, support for the project is increasing.

Another farmer, Abdellah Boudhira, has transitioned from modern intensive farming, using patented hybrid seeds, back to traditional organic practices using only heritage seeds, which he

believes taste better and have much higher nutritional value. He has also decided to sell directly to customers, as dependence on middlemen to access the market results in such low prices for producers and high costs for consumers. Boudhira's farm has garnered a following on social media as he attempts to raise his profile and encourage sustainable organic agriculture. He says that more people in Morocco are becoming interested in healthy organic food, and that, "Healthy food can be affordable for everybody if we farm wisely, and don't rely on chemical corporations." (Ken, Renske, Jens, & James, 2021).

The accomplishments of Boudhira demonstrate that farmers are capable of adapting their practices to be more sustainable. However, in order to do so, they require help in the form of funding, education, and upgraded facilities. Government policy should not just encourage the expansion of intensive agriculture but also provide substantial assistance to these small-scale farmers (Ken, Renske, Jens, & James, 2021).

Only two regions in Morocco provide food for the entire nation. Citrus and other veggies are even supplied by these two locations to Europe, Russia, the USA, Canada, and China. Large-scale agriculture in some areas has compacted the soil, causing water tables to nearly completely disappear in one region, and soil quality is also declining. Farmers can't produce the yields they need to keep up with rising input costs without heavily utilizing synthetic fertilizers (Jihane, 2018).

In order to encourage farmers to grow healthy food in a regenerative way, consumers must buy products from farmers at a price that will allow them to farm that way. Local farmers need that support so they can keep their land and keep working their land instead of selling it to move to overcrowded cities (Ken, Renske, Jens, & James, 2021).

Therefore, Morocco should prioritize agricultural development politically because it is essential for raising rural living standards and a key element of inclusive growth and reducing inequality. This is why we push for legislative measures including strong incentives for farmers to encourage regenerative practices and assist ensure farmer livelihoods, as well as government targets to protect and restore soil health (Alexandra & Griffin, 2016).

Conclusions

In actuality, ignoring climate change would be expensive, lead to great human suffering, and harm the environment, whereas switching to a greener economy would help a lot of people and ecosystems all over the world. Morocco is dealing with some issues related to soil degradation, drought, and climate change. The gap between large and small farms, as well as between the rich and the poor, calls for an innovative and varied approach to enhancing agriculture. If individuals were aware of the facts, they would be unable to continue to downplay the significance of global warming, and humanity as a whole would begin moving in the correct direction.

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Determination of Trace Elements Concentration in Date Palm Collected from some Arabian Countries

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Abstract

Plants have important roles in human lives. Many ethnic groups depend on plants for food and many other purposes from birth to death. This study aimed to determine trace elements concentration (Calcium (Ca), Potassium (K), Silicon (Si), Sulphur (S), Iron (Fe), Phosphorus (P), Bromine (Br), Rubidium (Rb), Copper (Cu), Zinc (Zn), Manganese (Mn), Chromium (Cr), and Lead (Pb) in Date Palm collected from some Arabian Countries (Egypt, Morocco, Sudan, and Tunisia). X-ray fluorescence (XRF) technique was applied to appreciate elements concentration. The results showed that Ca, K, Si, S, and Fe were found in all samples with approximately similar concentrations, whereas (P, Br, Rb, Cu, Zn, Mn, Cr, and Pb) were observed only in Sudanese and Moroccan samples. K and Ca showed maximum levels of about 2500 and 800 mg/kg respectively and Rb showed a minimum concentration of about 4 mg/kg. All Sudanese samples contain Br at low levels and most of the collected samples contain P, this is due to the huge uses of these two elements in fertilizers as bromide and triple superphosphate.

A mean comparison test was carried out on Ca, K, Si, S, and Fe in all samples, there are no statistical differences among their concentrations. Moreover, a correlation coefficient test was carried out on Br and P elements to find the relationship between which, there is no linear

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relationship between these elements.

Keywords: Date Palm, XRD, Trace Elements, Arabian Countries.

Discipline: Science and Engineering

Introduction

Plants have important roles in human lives. Many ethnic groups depend on plants for food and many other purposes from birth to death [1]. Fruits play an important role in the human diet as they supply vitamins and minerals, which are essential nutrients for human health. The date palm (*Phoenix dactylifera L.*, family *Arecaceae*) is one of the oldest cultivated trees in arid and semi-arid regions, it is one of the foremost imperative natural product crops in numerous parts of the world, especially in the Arabian countries where most consumers [2-4]. Worldwide date production has increased from (1.8 - 7.9 mtonsfromn ton in 1962 – 2014) respectively. Egypt, Sudan, and Tunisia were among the top ten date-producing countries in the world in 2005. Tunisia was among the top five date-exporting countries whereas Morocco was among the top five date-importing countries in 2004 [4, 5]. Date natural products have a great significance from both a nutritional and therapeutic which are wealthy sources of carbohydrates, minerals, vi mins, and fibers. In some categories, the carbohydrate substance of the natural products comes to up to 88% and such natural products are considered high-energy nourishment sources [6].

Date palm fruits contain some kinds of vitamins C, B1 and B2 [7] wherefore, are considered solid nourishment with anticancer, antiviral and antioxidant activities [8-10]. Their antioxidant effectiveness is due to the existence of flavonoids in their fruits, and they are sometimes exhorted for pregnant ladies before and after giving birth due to their beneficial nutrients and pleasant taste. Dates include ratable quantities of elements such as K, Na, Ca, and P that are deemed as macro-element, however, Be, Fe, F, I, Co, Cu, Mn, , Mg, Mo, Se, S, and Zn are classified as micro-elements [9]. Also contain transition metals or metalloids (As, Cd, Cr, and Ni) which are considered human carcinogens at broad exposure [9]. One commonly used method for characterizing trace elements in the plant is the energy dispersive X-ray

fluorescence (ED-XRF), this technique allows for easy, rapid and simultaneous detection of elements between Na and U with a large dynamic range (mg/kg to µg/kg). Consequently, it is used as qualitative analysis in 30 s, or so in a variety of fields, including environmental research, medicine, geology, and archaeology. Generally, XRF techniques have advantages such as; simplicity of spectra and sample preparation, analyte chemical states do not affect the spectrum places, non-destructive analysis, applicability over a wide range of concentrations, the goodness of precision and accuracy, and measurement usability for solid, powdered and liquid samples [11-13].

On the other hand, they also have some disadvantages such as limitation of X-ray sample penetration (0.01 - 0.1 mm), sensitivity limitation for the light elements (below Na) though the modern instruments can analyze till C, matrices interference effects, detection limits are only modest, and expensiveness of instrumentation [11].

This work was done to appreciate trace elements concentration in the *Phoenix dactylifera*.

Material and Methods

Sample Collection

Fifteen samples of dates of different varieties and origins were analyzed, seven from Sudan, four from Morocco, two from Egypt and two from Tunisia. All samples were analyzed in the Tamer one. Botanical identification and authentication of the Sudanese collected species were done by the Medicinal and Aromatic Plants Research Institute (MAPRI) - National Centre for Research-Sudan. Common, scientific names and collection areas of study plants are given in Table 1.

Table 1: A brief review of about the studied plant.

Botanical Name	**Local Name**	**Family**	**Collection Area**	**Part used**
Phoenix dactylifera	**(Ar) Tamr**	**Arecaceae**	**====**	**Fruits**
S_1	*Berkawi*	**Arecaceae**	Sudan	Fruits
S_2	*Gondela*	**Arecaceae**	Sudan	Fruits
S_3	*Jawa*	**Arecaceae**	Sudan	Fruits
S_4	*Tamouda*	**Arecaceae**	Sudan	Fruits
S_5	*Agwa*	**Arecaceae**	Sudan	Fruits

S_6	*Kulum*	**Arecaceae**	Sudan	Fruits
S_7	*Mishrig*	**Arecaceae**	Sudan	Fruits
S_8	*Deglet Nour*	**Arecaceae**	Morocco	Fruits
S_9	*Mejhol*	**Arecaceae**	Morocco	Fruits
S_{10}	*Aziza*	**Arecaceae**	Morocco	Fruits
S_{11}	*Aguelid*	**Arecaceae**	Morocco	Fruits
S_{12}	*Siwi*	**Arecaceae**	Egypt	Fruits
S_{13}	*Umm alfirakh*	**Arecaceae**	Egypt	Fruits
S_{14}	*Kentishi*	**Arecaceae**	Tunisia	Fruits
S_{15}	*Ollegi*	**Arecaceae**	Tunisia	Fruits

Sample Preparation

2.2.1. Sample Drying and Homogenizing

Samples were thoroughly washed in running tap water to eliminate any potential contamination from the date surface, and then properly dried. Seeds were separated from date. Samples were dried at room temperature, then put in the oven at 110 centigrade to evaporate any moisture in them for one hour; Aliquots of the plant material were ground and homogenized by a mortar and sieving. The resulting fine powder was used for further analysis.

Sample Preparation for ED-XRF Analysis

One gram of the powder sample was pressed (15 ton/cm^2). A standard reference material (IAEA-V-10 Hay powder) was used for quality control purposes and has been treated in the same manner.

Sample Analysis

XRF Measurement

The energy dispersive X-Ray Fluorescence (EDXRF) spectrometer, equipped with Si (Li) detector and radioisotope ^{109}Cd with energy 22.1 keV as a primary source was used to measure these elements (Ca, K, Si, S, Fe, P, Br, Rb, Cu, Zn, Mn, Ti Cr, Ni and Pb) in the plant samples. This technique also has a disadvantage that could be affected by matrix constituents, which is expected to enhance the concentrations of some elements.

Results and Discussions

From Table 3.1; Ca, K, Si, S and Fe were found in all samples with approximately similar concentrations, except some samples showed distinctly different trends from the rest. K and Ca showed maximum levels of about 2500 (S_5) and 800 (S_{13}) mg/kg respectively, and Rb showed the most minimum concentration of about 4 mg/kg (S_3). P, Br, Rb, Cu, Zn, Mn, Cr and Pb were observed in some Sudanese and Moroccan samples rather than Egyptian and Tunisian samples. All Sudanese samples contain bromide at low levels (13.6 – 30.8 mg/kg), due to its huge uses for agricultural purposes as fertilizer. Bromine is toxic at higher levels (intakes >50-100 mg/day) [14]. Samples (2, 3, 4, 6, 7, 8, 9, 11 and 13) contain different amounts of phosphorus, this also could be due to the use of fertilizers, with high amounts as triple superphosphate (48% P_2O_5) [15]. There is optical interference between Pb and S elements making Pb appear just in S_{10}, the reason for this interference is M-α line of Pb 'overlaps' with the K-α line of S at 2.346 and 2.307 keV respectively [16]. Also expected interference takes place between K-β of Cr (5.946 keV) and K-α of Mn (5.899 keV) and between K-α of P and K-α of Cu (6.155 and 6.167 keV) respectively, this makes Cu found in six samples rather than the others [17, 18].

Comparing the concentrations of the elements among the date species from the same country, it can be observed that K showed the higher concentrations, while Fe showed the lower concentrations as can be seen in Figures 1, 2, 3, and 4.

Table 2: Elements concentrations of the samples under study in mg/kg.

Sample	Ca	K	Si	S	Fe	P	Br	Rb	Cu	Zn	Mn	Cr	Pb
S_1	647.5	2400	290	110.0	60.7	----	19.3	7.9	----	----	----	----	----
S_2	592.9	2473	178	99.9	57.6	59.5	22.4	9.6	7.14	----	----	----	----
S_3	758.9	2347	146	97.9	66.0	34.9	29.5	3.5	7.07	8.4	----	----	----
S_4	747.1	2314	237	115.6	38.7	25.6	14.1	----	----	8.3	----	----	----
S_5	797.3	2177	313	96.9	94.7	----	14.6	5.9	----	----	----	----	----
S_6	400.1	2412	276	186.9	62.8	65.2	13.6	----	38.4	43.8	----	----	----
S_7	566.8	2420	258	107.2	28.3	79.5	30.8	----	10.1	----	----	----	----
S_8	506.2	2161	264	105.0	322.4	49.8	----	7.3	17.7	----	17.2	19.0	----
S_9	588.5	2392	291	112.2	58.3	58.1	----	----	55.1	52.0	----	----	----
S_{10}	439.9	2277	357	242.9	49.4	----	----	----	----	----	----	----	26.6

S_{11}	570.2	2469	232	126.8	41.4	61.2	----	----	----	----	----	----	----
S_{12}	661.5	2301	272	112.7	60.8	----	----	----	----	----	----	----	----	----
S_{13}	430.5	2508	272	133.1	62.4	93.8	----	----	----	----	----	----	----	----
S_{14}	682.9	2187	435	140.8	54.6	----	----	----	----	----	----	----	----	----
S_{15}	604.1	2263	419	133.7	80.7	----	----	----	----	----	----	----	----	----

No significant differences were observed generally between the concentrations of the elements and in particular, Si and S were found to be in medium levels while Fe was observed at lower levels, in all samples. The mean comparison has been performed using the Kruskal-Walis test as an alternative to ANOVA due to normality issues. The P. values above which are greater than 0.05 illustrate insignificant differences between the four countries among the concentration of all five elements as shown in Table 2. This conclusion could be also obtained from Figure 3.5, and all four locations showed slight differences in the concentrations of these main elements.

Table 3: P-values of the comparison of some elements' mean concentrations between the different countries.

Elements	**Fe**	**S**	**Si**	**K**	**Ca**
P-value	0.926	0.233	0.140	0.347	0.339

The correlation between the concentration of Br and P elements was performed to test their association with each other since they are said to be originated from fertilizers residues. From Table 3**.** the P- value was greater than 0.05 (0.325), therefore, there is no linear relationship between the concentrations of Br and P elements.

Table 4: Correlation coefficient and P-values of the elements (Br, P) concentrations.

		Br	P
Br	**Pearson Correlation**	1	0.439
	Sig. (2-tailed)		0.325
P	**Pearson Correlation**	0.439	1
	Sig. (2-tailed)	0.325	

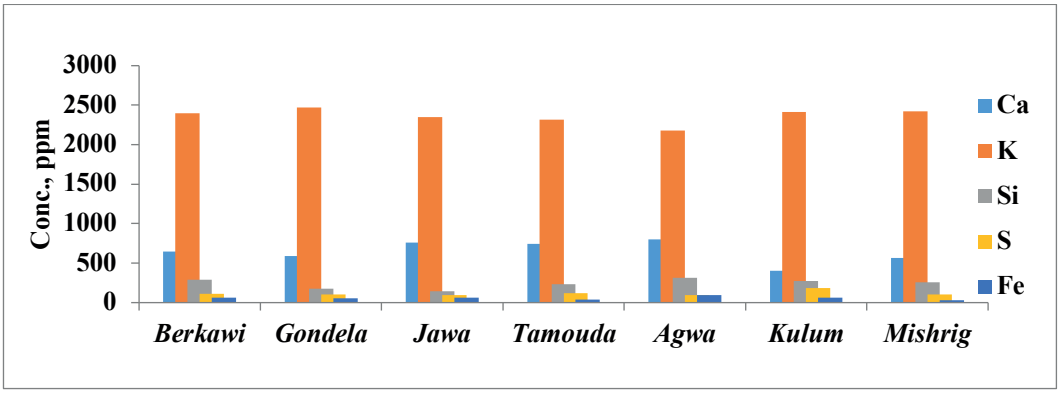

Figure 1: The elements concentration of Sudanese samples.

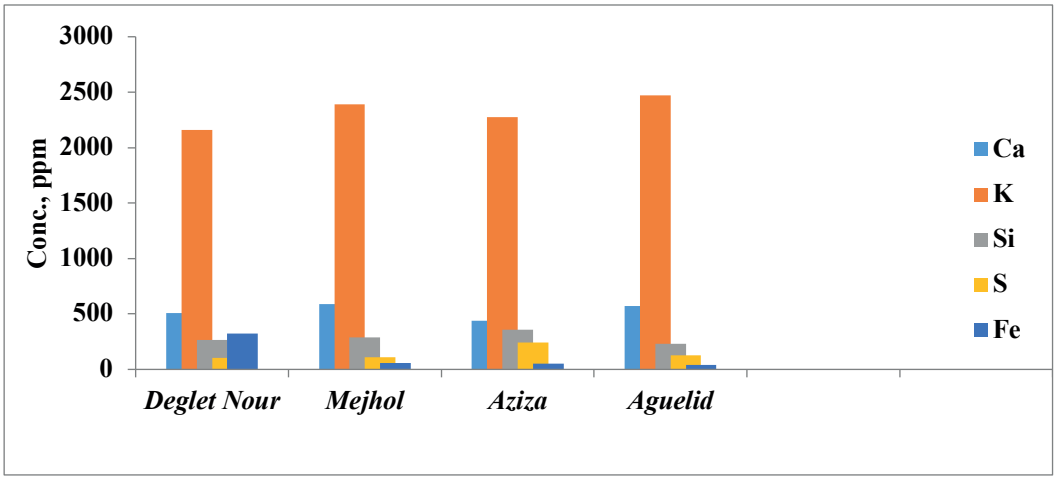

Figure 2: The elements concentration of Moroccan samples.

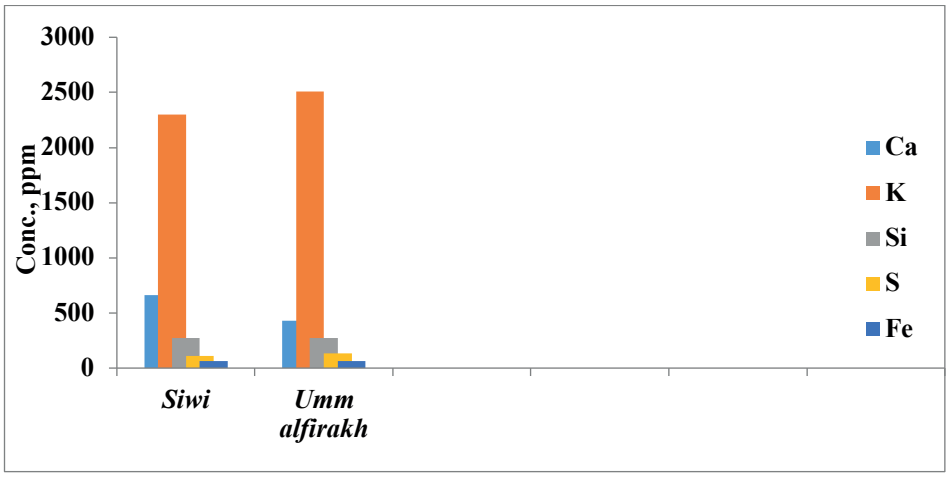

Figure 3: The elements concentration of Egyptian samples

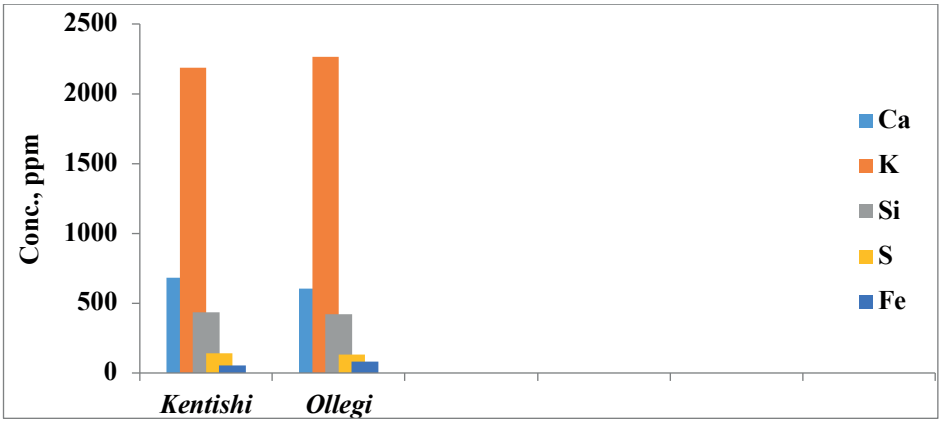

Figure 4: The elements concentration of Tunisian samples

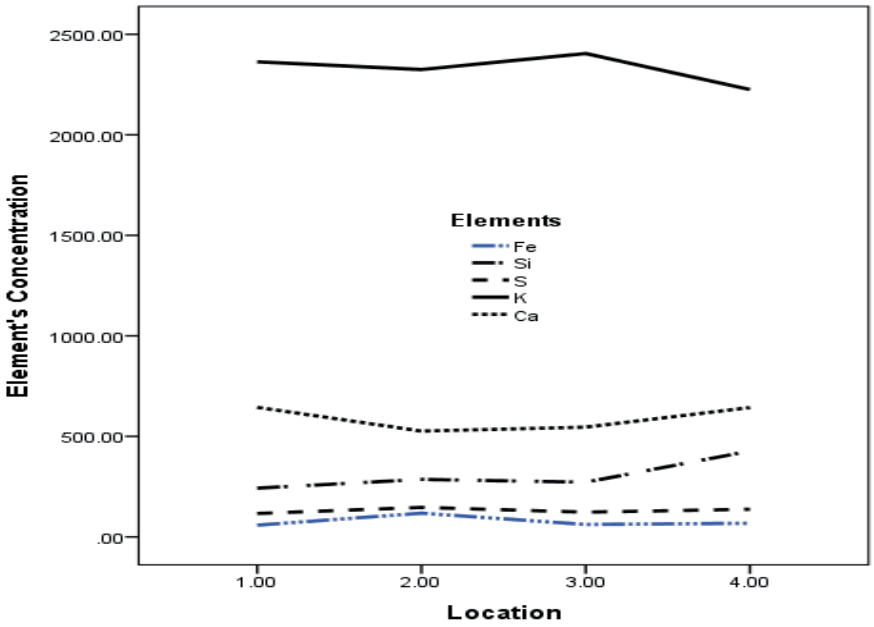

Figure 5: The mean comparison of the element concentrations of all countries

Conclusions

- Five elements (K, Ca, S, Si and P) were found in all samples with approximately similar concentrations. Therefore, no significant differences were observed generally between the concentrations of these elements.
- K and Ca showed maximum levels of about 2500 (S_5) and 800 (S_{13}) mg/kg respectively.
- Rb showed a minimum concentration of about 4 mg/kg (S_3).
- Some elements were observed in some Sudanese and Moroccan samples rather than Egyptian and Tunisian samples (P, Br, Rb, Cu, Zn, Mn, Cr and Pb). All Sudanese samples contain bromide at low levels (13.6-30.8 mg/kg). Cu was found in six samples rather than the others due to optical interferences.
- Statistically there is no linear relationship between the concentrations of Br and P elements.

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Soliton Solitons of the Nonlinear Duffing Equation using Newly φ^6-Model Expansion Approach

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Abstract

In this paper, we use the the φ^6-model expansion method to construct the traveling wave solutions for the nonlinear Duffing equation, the equation that describes the motion of a classical object in a double well potential is described by the Duffing equation. This equation has the potential to become chaotic. The method of φ^6-model expansion enables the explicit retrieval of a wide variety of solution types, such as bright, singular, periodic, and combined singular soliton solutions. Kink-type solitons, also known as topological solitons in the context of water waves, are another type of solution that can be explicitly retrieved. The results of this study might enhance the nonlinear dynamical properties of the equation. A practical and efficient method for solving a sizable class of nonlinear partial differential equations is proposed by the method. The dynamical features of the data are explained and highlighted using interesting graphs.

Keywords: Nonlinear Duffing model, Jacobi elliptic solutions, φ^6-model expansion approach, kink soliton.

Introduction

Partial differential equations (PDEs) first appeared in the study of surfaces in geometry [1-5] and a wide range of problems in mechanics. In the late 19th century, prominent mathematicians from all over the world became actively interested in the study of a broad range of problems

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caused by partial differential equations. Partial differential equations frequently emerge in the mathematical analysis of a variety of problems in science and engineering and describe many fundamental natural principles, which was the primary driving force behind this work [6]. It is now incredibly beneficial to look for precise answers to the both nonlinear evolution equations and partial differential equations NLEEs using a variety of techniques, and there are numerous effective techniques, such the inverse scattering transform approach [7], the Homoclinic technique [8], the sinh-Gordon function method [9], the generalized exponential rational function method [10], the auxiliary equation method [11], An alternative method [12], the Bernoulli sub-equation function method [13, 14], the sub-equation analytical method [15], the modified sub-equation method [16], the auto-Bäcklund transformation method [17] and so on.

This study focuses on the nonlinear Duffing equation, the equation that describes the motion of a classical object in a double well potential is described by the Duffing equation. This

Eq.(10) has the potential to become chaotic. The equation depicts a stiff spring when $n > 0$, as well as a soft spring for $n < 0$ [18]. The equation has been investigated via many direct methods. Among them are; Aminah used the sine-cosine function method [18] to construct the periodic wave solutions. The exact particular solutions are obtained including periodic solutions using the $\left(\frac{\Phi(\xi)}{2}\right)$ −expansion method [19] by Jalil et al. Balaji solved the Duffing equation for integral and non-integral forcing terms [20]. The first integral method [21]. The Duffing model is studied in this research using the newly developed φ^6–model expansion method [22-25]), which results in the restoration of optical solitary wave solutions.

The plan for this work is provided below. In Section 2, a presentation of the φ^6-model expansion method will be provided. The Duffing equation will be developed using the φ6-approach in section 3 to provide new traveling wave solutions to the Duffing equation. Moreover, the associated 3D, 2D and density graphs clearly illustrate the physical structure of the traveling wave solution. In section 4, the soliton solutions' physical dynamics are examined, and in section 5, conclusions are reached.

Description of The Method

According to [22-25], the steps involves for the φ^6-model expansion technique are given as:

Step-1: Assuming the nonlinear evolution equation (NLEE) for $G = G(x,t)$ is in the form.

$$H(G, G_x, G_t, G_{xx}, G_{xt}, \dots) = 0, \qquad (1)$$

here H is a polynomial of $G(x,t)$ which involves highest order partial derivatives and its nonlinear terms.

Step-2: By using the wave transformation

$$Q(x,t) = Q(\zeta), \quad \zeta = x - vt, \qquad (2)$$

where w represents wave speed and Eq.(1) can be converted into the nonlinear ordinary differential equation shown below.

$$\Omega(G, G', GG', G'', \dots) = 0, \quad (3)$$

where the derivatives with respect to ζ are shown by prime. **Step-3**: Suppose that the formal solution to Eq.(3) exists:

$$G(\zeta) = \sum_{j=0}^{2M} \alpha_j A^j(\zeta), \qquad (4)$$

M can be gotten using the balancing rule, $\alpha_j (j = 0,1,2, \dots, M)$ are to be determined constants and $A(\zeta)$ satisfies the auxiliary NLODE;

$$A'^2(\zeta) = h_0 + h_2 A^2(\zeta) + h_4 A^4(\zeta) + h_6 A^6(\zeta), \qquad (5)$$

$$A''(\zeta) = h^2 A(\zeta) + 2h_4 A^3(\zeta) + 3h_6 A^5(\zeta),$$

here $h_j (j = 0,2,4,6)$ are real constants that will be found later.

Step-4: It is known that the solution to Eq.(5) is given as;

$$U(\zeta) = \frac{P(\zeta)}{\sqrt{fP^2(\zeta)+g}}, \qquad (6)$$

$P(\zeta)$ is the Jacobi elliptic equation solution, provided that $0 < fP^2(\zeta) + g$

$$P'^2(\zeta) = l_0 + l_2 P^2(\zeta) + l_4 P^4(\zeta), \quad (7)$$

where $l_j (j = 0,2,4)$ are unknown constants to be determined, g and f are given by

$$f = \frac{h_4(l_2 - h_2)}{(l_2 - h_2)^2 + 3l_0 l_4 - 2l_2(l_2 - h_2)}, \quad (8)$$

$$g = \frac{3l_0 h_4}{(l_2 - h_2)^2 + 3l_0 l_4 - 2l_2(l_2 - h_2)},$$

under the restricted condition

$$h_4^2(l_2 - h_2)[9l_0 l_4 - (l_2 - h_2)(2l_2 + h_2)] + 3h_6[-l_2^2 + h_2^2 + 3l_0 l_4]^2 = 0. (9)$$

Step-5: The Jacobi elliptic solutions of Eq.(7) can be calculated when $0 < m < 1$, the exact solutions of Eq.(1) can be derived by substituting Eq.(6) and Eq.(7) into Eq.(4).

Function	$m \to 1$	$m \to 0$	Function	$m \to 1$	$m \to 0$
$sn(\zeta, m)$	$tanh(\zeta)$	$sin(\zeta)$	$ds(\zeta, m)$	$csch(\zeta)$	$csc(\zeta)$
$cn(\zeta, m)$	$sech(\zeta)$	$cos(\zeta)$	$sc(\zeta, m)$	$sinh(\zeta)$	$tan(\zeta)$
$dn(\zeta, m)$	$sech(\zeta)$	1	$sd(\zeta, m)$	$sinh(\zeta)$	$sin(\zeta)$
$ns(\zeta, m)$	$coth(\zeta)$	$csc(\zeta)$	$nc(\zeta, m)$	$cosh(\zeta)$	$sec(\zeta)$
$cs(\zeta, m)$	$csch(\zeta)$	$cot(\zeta)$	$cd(\zeta, m)$	1	$cos(\zeta)$

Application of the Proposed Method to the Duffing Equation

The φ^6-model expansion method, which was explained in Part 2, will be used in this section to retrieve the exact solitary wave solutions of the nonlinear Duffing equation.

$$G_{tt} + \delta G + nG^3 = 0, \quad (10)$$

When real constants δ and n are used. The motion of a classical object in a double well potential is described by the Duffing equation. This equation has the potential to become chaotic. The equation depicts a stiff spring when $n > 0$, as well as a soft spring for $n < 0$. Eq.(10) is reduced to the following ODE using the traveling wave transformation $G(x,t) = G(\zeta) = G(x - vt)$:

$$v^2 G'' + nG^3 + \delta G = 0. \quad (11)$$

where $M = 1$ is obtained from the balance principle between G'' and G^3; as a result, the solution form can be expressed as

$$P(\zeta) = \alpha_0 + \alpha_1 A(\zeta) + \alpha_2 A^2(\zeta), \quad (12)$$

where α_0, α_1 and α_2 are constants to be determined.

We obtain the following algebraic equations by substituting Eq.(12) along with Eq.(5) into Eq.(11) and setting the coefficients of all powers of $A^j(\zeta)$, $j = 0,1, \ldots ,6$ to be equal to zero

$$\begin{aligned}
&A^0(\zeta): \alpha_0\delta + 2\alpha_2 h_0 v^2 + \alpha_0^3 n = 0,\\
&A^1(\zeta): \alpha_1\delta + \alpha_1 h_2 v^2 + 3\alpha_0^2\alpha_1 n = 0,\\
&A^2(\zeta): \alpha_2\delta + 4\alpha_2 h_2 v^2 + 3\alpha_0\alpha_1^2 n + 3\alpha_0^2\alpha_2 n = 0,\\
&A^3(\zeta): 2\alpha_1 h_4 v^2 + \alpha_1^3 n + 6\alpha_0\alpha_2\alpha_1 n = 0,\\
&A^4(\zeta): 6\alpha_2 h_4 v^2 + 3\alpha_0\alpha_2^2 n + 3\alpha_1^2\alpha_2 n = 0,\\
&A^5(\zeta): 3\alpha_1 h_6 v^2 + 3\alpha_1\alpha_2^2 n = 0,\\
&A^6(\zeta): 8\alpha_2 h_6 v^2 + \alpha_2^3 n.
\end{aligned} \quad (13)$$

the following solutions can be obtained after solving the above resulting system :

$$\alpha_0 = 0, \quad \alpha_2 = 0, \quad \alpha_1 = \frac{i\sqrt{2}\sqrt{h_4}v}{\sqrt{n}}, \quad (14)$$

$$h_2 = -\frac{\delta}{v^2}, \quad h_6 = 0,$$

the following exact solutions of Eq.(10) can be derived with the help of Eqs. (6), (12) and (14) along with the Jacobi elliptic functions in the above table

1. If $l_0 = 1$, $l_2 = -(1 + m^2)$, $l_4 = m^2$, $0 < m < 1$, then $P(\zeta) = sn(\zeta, m)$ or $P(\zeta) =$

$cd(\zeta, m)$, and we have

$$G_{1,0}(x,t) = \alpha_1\left(\frac{sn(\zeta,m)}{\sqrt{f(sn(\zeta,m))^2+g}}\right), \qquad (15)$$

or

$$G_{1,1}(x,t) = \alpha_1\left(\frac{cd(\zeta,m)}{\sqrt{f(cd(\zeta,m))^2+g}}\right), \qquad (16)$$

such that $\zeta = x - vt$ and f and g in Eq. (8) are given by

$$f = \frac{h_4 v^2\left((m^2+1)v^2-\delta\right)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = -\frac{3h_4 v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the restriction condition

$$h_4^2\left(-m^2+\frac{\delta}{v^2}-1\right)\left(9m^2-\left(2(-m^2-1)-\frac{\delta}{v^2}\right)\left(-m^2+\frac{\delta}{v^2}-1\right)\right)=0.$$

If $m \to 1$, then the kink soliton is obtained

$$G_{1,2}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\tanh(tv-x)}{\sqrt{n}\sqrt{\frac{h_4 v^2\left((2v^2-\delta)\tanh^2(tv-x)-3v^2\right)}{v^4-\delta^2}}}, \qquad (17)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}-2\right)\left(9-\left(-\frac{\delta}{v^2}-4\right)\left(\frac{\delta}{v^2}-2\right)\right)=0.$$

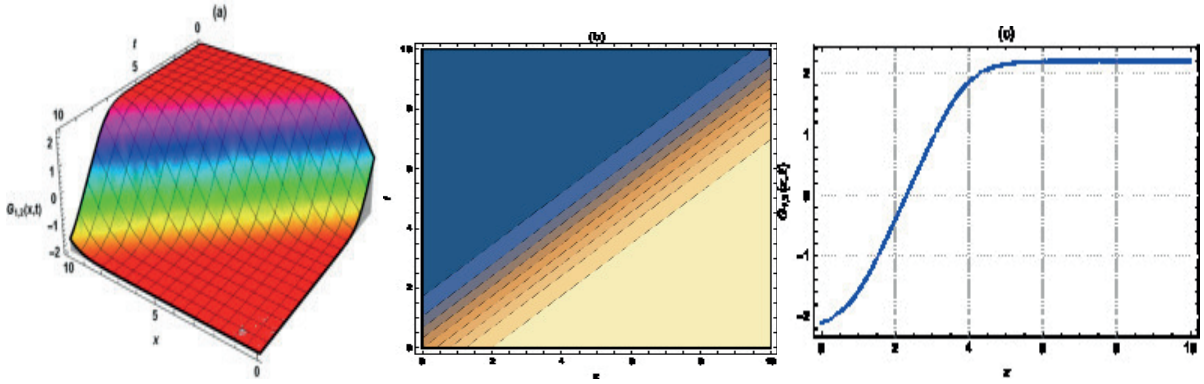

Figure 1: The numerical simulations corresponding to $|G_{1,2}|$ *given by Eq.(17), for* $m = 1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta = 0.1, v = 1.15, n = 0.5, h_4 = 0.3$.

If $m \to 0$, then the periodic solution is obtained

$$G_{1,3}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\sin(tv-x)}{\sqrt{n}\sqrt{\frac{h_4 v^2\left((v^2-\delta)\sin^2(tv-x)-3v^2\right)}{v^4-\delta^2}}}, \qquad (18)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}-1\right)\left(-\left(-\frac{\delta}{v^2}-2\right)\left(\frac{\delta}{v^2}-1\right)\right)=0.$$

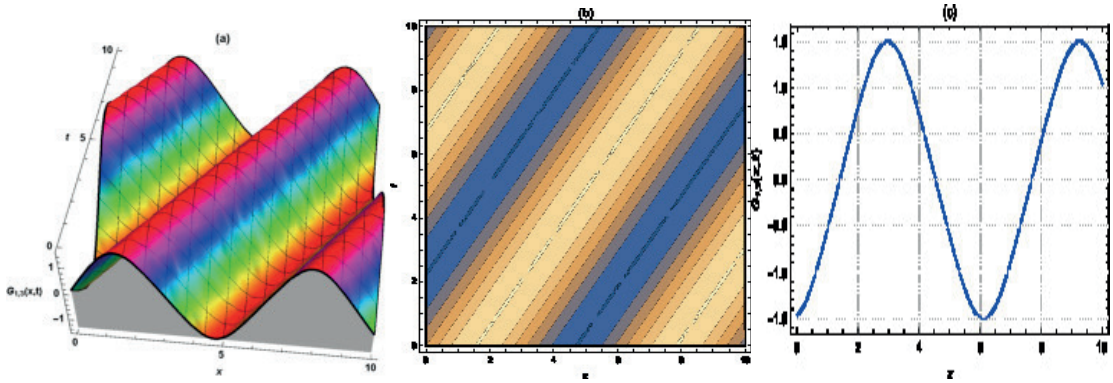

Figure 2: The numerical simulations corresponding to $|G_{1,3}|$ *given by Eq.(18), for* $m = 1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta = 0.2, v = 0.7, n = 0.15, h_4 = 0.03$.

2. If $l_0 = 1 - m^2, l_2 = 2m^2 - 1, l_4 = -m^2, 0 < m < 1$, then $P(\zeta) = cn(\zeta, m)$ therefore

$$G_{2,1}(x,t) = \alpha_1\left(\frac{cn(\zeta,m)}{\sqrt{f(cn(\zeta,m))^2+g}}\right), \qquad (19)$$

where f and g are determined by

$$f = -\frac{h_4v^2(\delta+(2m^2-1)v^2)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = \frac{3h_4(m^2-1)v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(2m^2+\frac{\delta}{v^2}-1\right)\left(-\left(2(2m^2-1)-\frac{\delta}{v^2}\right)\left(2m^2+\frac{\delta}{v^2}-1\right)-9(1-m^2)m^2\right)=0$$

If $m \to 1$, then the bright soliton is retrieved

$$G_{2,2}(x,t) = \frac{i\sqrt{h_4}v\,\mathrm{sech}(tv-x)}{\sqrt{n}\sqrt{-\frac{h_4v^2\,\mathrm{sech}^2(tv-x)}{2v^2-2\delta}}} \qquad (20)$$

provided that

$$h_4^2\left(\frac{\delta}{v^2}+1\right)\left(-\left(2-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+1\right)\right)=0.$$

If $m \to 0$, then the periodic solution is obtained

$$G_{2,3}(x,t) = \frac{i\sqrt{2}\sqrt{h_4}v\cos(tv-x)}{\sqrt{n}\sqrt{\frac{h_4v^2\left((v^2-\delta)\cos^2(tv-x)-3v^2\right)}{v^4-\delta^2}}}, \qquad (21)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}-1\right)\left(-\left(-\frac{\delta}{v^2}-2\right)\left(\frac{\delta}{v^2}-1\right)\right)=0.$$

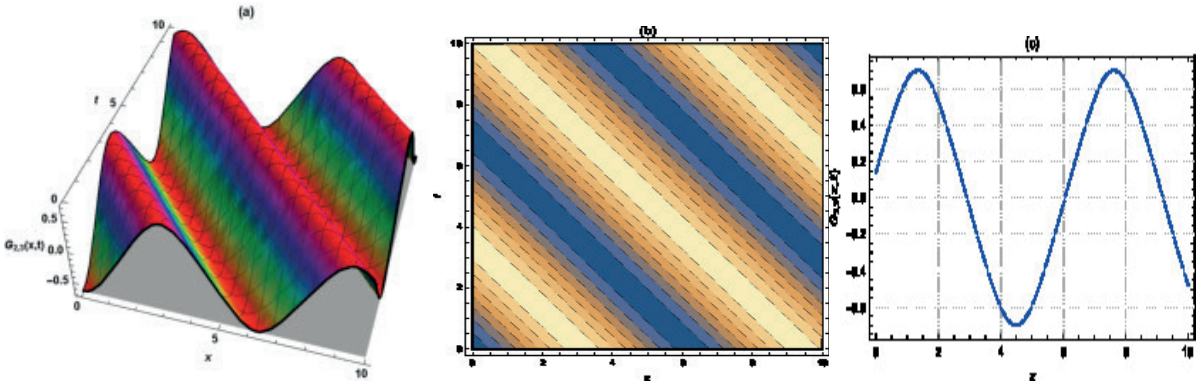

Figure 3: The numerical simulations corresponding to $|G_{2,3}|$ *given by Eq.(21), for* $m = 1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta = 0.3, v = -0.9, n = 1.2, h_4 = 4$.

3. If $l_0 = m^2 - 1, l_2 = 2 - m^2, l_4 = -1, 0 < m < 1$, then $P(\zeta) = dn(\zeta, m)$ which gives

$$G_{3,1}(x,t) = \alpha_1 \left(\frac{dn(\zeta,m)}{\sqrt{f(dn(\zeta,m))^2 + g}} \right), \qquad (22)$$

where f and g are determined by

$$f = \frac{h_4 v^2 \left((m^2-2)v^2 - \delta \right)}{(m^4 - m^2 + 1)v^4 - \delta^2},$$

$$g = -\frac{3h_4(m^2-1)v^4}{(m^4 - m^2 + 1)v^4 - \delta^2},$$

under the restriction condition

$$h_4^2 \left(-m^2 + \frac{\delta}{v^2} + 2 \right) \left(-\left(2(2 - m^2) - \frac{\delta}{v^2} \right) \left(-m^2 + \frac{\delta}{v^2} + 2 \right) - 9(m^2 - 1) \right) = 0$$

If $m \to 0$, then the rational solution is obtained

$$G_{3,3}(x,t) = \frac{i\sqrt{2}\sqrt{h_4}v}{\sqrt{n}\sqrt{\frac{3h_4v^4}{v^4-\delta^2} - \frac{h_4v^2(\delta+2v^2)}{v^4-\delta^2}}}, \qquad (23)$$

such that

$$h_4^2 \left(\frac{\delta}{v^2} + 2 \right) \left(9 - \left(4 - \frac{\delta}{v^2} \right) \left(\frac{\delta}{v^2} + 2 \right) \right) = 0.$$

4. If $l_0 = m^2,\ l_2 = -(1+m^2),\ l_4 = 1,\ 0 < m < 1,$ then $P(\zeta) = ns(\zeta, m)$ or $P(\zeta) = dc(\zeta, m)$ then

$$G_{4,0}(x,t) = \alpha_1\left(\frac{ns(\zeta,m)}{\sqrt{f(ns(\zeta,m))^2+g}}\right), \qquad (24)$$

or

$$G_{4,1}(x,t) = \alpha_1\left(\frac{dc(\zeta,m)}{\sqrt{f(dc(\zeta,m))^2+g}}\right), \qquad (25)$$

where f and g are given by

$$f = \frac{h_4 v^2\left((m^2+1)v^2-\delta\right)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = -\frac{3h_4 m^2 v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(-m^2+\frac{\delta}{v^2}-1\right)\left(9m^2-\left(2(-m^2-1)-\frac{\delta}{v^2}\right)\left(-m^2+\frac{\delta}{v^2}-1\right)\right)=0.$$

If $m \to 1$, then the dark singular solution is obtained

$$G_{4,2}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\coth(tv-x)}{\sqrt{n}\sqrt{\frac{h_4 v^2\left((2v^2-\delta)\coth^2(tv-x)-3v^2\right)}{v^4-\delta^2}}} \qquad (26)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}-2\right)\left(9-\left(-\frac{\delta}{v^2}-4\right)\left(\frac{\delta}{v^2}-2\right)\right)=0$$

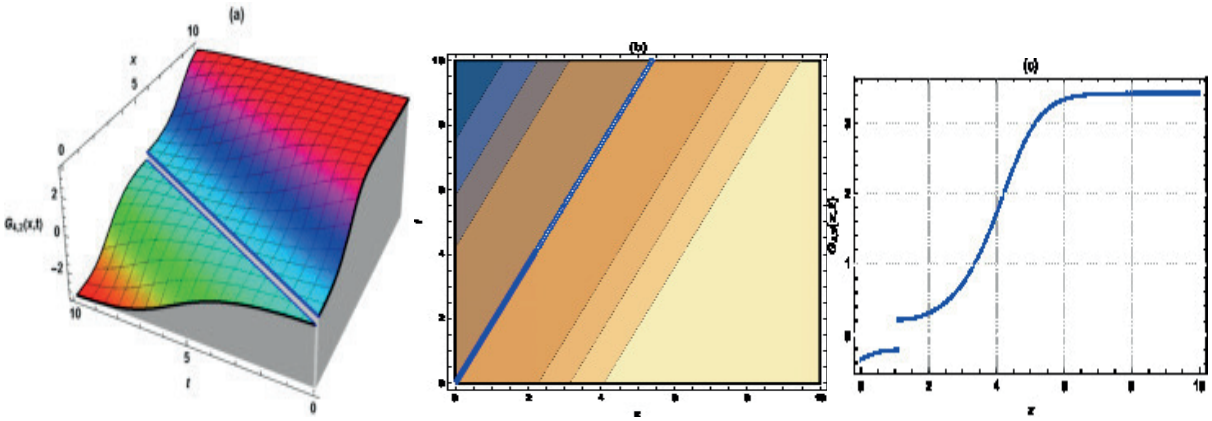

Figure 4: The numerical simulations corresponding to $|G_{4,2}|$ *given by Eq.(26), for* $m = 1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta = -0.295, v = 0.54, n = 0.1, h_4 = 3.6$.

If $m \to 0$, then the periodic solution is obtained

$$G_{4,3}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\csc(tv-x)}{\sqrt{n}\sqrt{\frac{h_4 v^2 \csc^2(tv-x)}{\delta+v^2}}}, \qquad (27)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}-1\right)\left(-\left(-\frac{\delta}{v^2}-2\right)\left(\frac{\delta}{v^2}-1\right)\right)=0.$$

5. If $l_0 = -m^2, l_2 = 2m^2 - 1, l_4 = 1 - m^2, 0 < m < 1$, then $P(\zeta) = nc(\zeta, m)$ and we have

$$G_{5,1}(x,t) = \alpha_1\left(\frac{nc(\zeta,m)}{\sqrt{f(nc(\zeta,m))^2+g}}\right), \qquad (28)$$

where f and g are given by

$$f = -\frac{h_4 v^2(\delta+(2m^2-1)v^2)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = \frac{3h_4 m^2 v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(2m^2+\frac{\delta}{v^2}-1\right)\left(-\left(2(2m^2-1)-\frac{\delta}{v^2}\right)\left(2m^2+\frac{\delta}{v^2}-1\right)-9(1-m^2)m^2\right)=0.$$

If $m \to 1$, then the singular solitary wave solution is obtained

$$G_{5,2}(x,t) = \frac{i\sqrt{2}\sqrt{h_4}v\cosh(tv-x)}{\sqrt{n}\sqrt{-\frac{h_4v^2\left((\delta+v^2)\cosh^2(tv-x)-3v^2\right)}{v^4-\delta^2}}}, \quad (29)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}+1\right)\left(-\left(2-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+1\right)\right)=0.$$

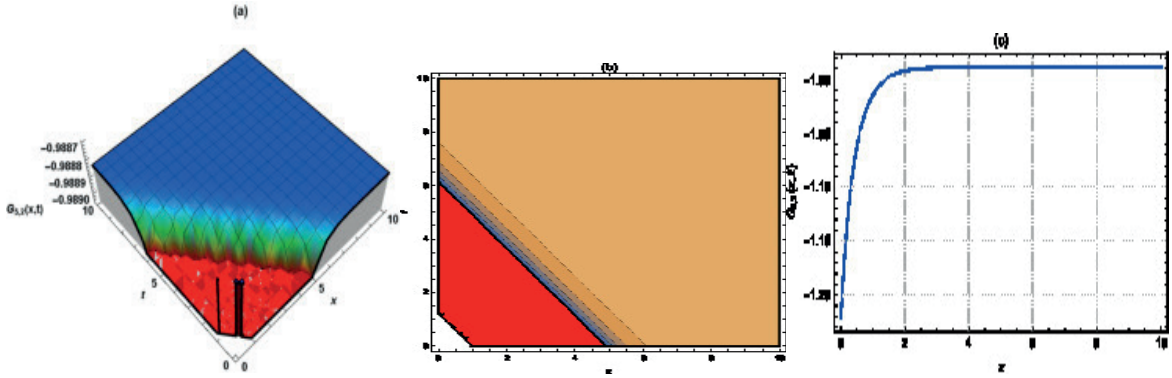

Figure 5: The numerical simulations corresponding to $|G_{5,2}|$ given by Eq.(29), for $m = 1$; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for $\delta = 0.2, v = -0.8, n = 0.9, h_4 = 1.8$.

If $m \to 0$, then the periodic solution is obtained

$$G_{5,3}(x,t) = \frac{i\sqrt{2}\sqrt{h_4}v\sec(tv-x)}{\sqrt{n}\sqrt{\frac{h_4v^2\sec^2(tv-x)}{\delta+v^2}}}, \quad (30)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}-1\right)\left(-\left(-\frac{\delta}{v^2}-2\right)\left(\frac{\delta}{v^2}-1\right)\right)=0.$$

6. If $l_0 = -1, l_2 = 2-m^2, l_4 = -(1-m^2), 0 < m < 1$, then $P(\zeta) = nd(\zeta, m)$ and we have

$$G_6(x,t) = \alpha_1\left(\frac{nd(\zeta,m)}{\sqrt{f(nd(\zeta,m))^2+g}}\right), \quad (31)$$

where f and g are given by

$$f = \frac{h_4 v^2\left((m^2-2)v^2-\delta\right)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = \frac{3h_4 v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(-m^2+\frac{\delta}{v^2}+2\right)\left(-\left(2(2-m^2)-\frac{\delta}{v^2}\right)\left(-m^2+\frac{\delta}{v^2}+2\right)-9(m^2-1)\right)=0.$$

7. If $l_0 = 1$, $l_2 = 2-m^2$, $l_4 = 1-m^2$,$0 < m < 1$, then $P(\zeta) = sc(\zeta, m)$, and we have

$$G_{7,1}(x,t) = \alpha_1\left(\frac{sc(\zeta,m)}{\sqrt{f(sc(\zeta,m))^2+g}}\right), \qquad (32)$$

where f and g are given by

$$f = \frac{h_4 v^2\left((m^2-2)v^2-\delta\right)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = -\frac{3h_4 v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(-m^2+\frac{\delta}{v^2}+2\right)\left(9(1-m^2)-\left(2(2-m^2)-\frac{\delta}{v^2}\right)\left(-m^2+\frac{\delta}{v^2}+2\right)\right)=0.$$

If $m \to 1$, then the soliton solution is retrieved

$$G_{7,2}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\sinh(tv-x)}{\sqrt{n}\sqrt{-\frac{h_4 v^2\left((\delta+v^2)\sinh^2(tv-x)+3v^2\right)}{v^4-\delta^2}}}, \qquad (33)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}+1\right)\left(-\left(2-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+1\right)\right).$$

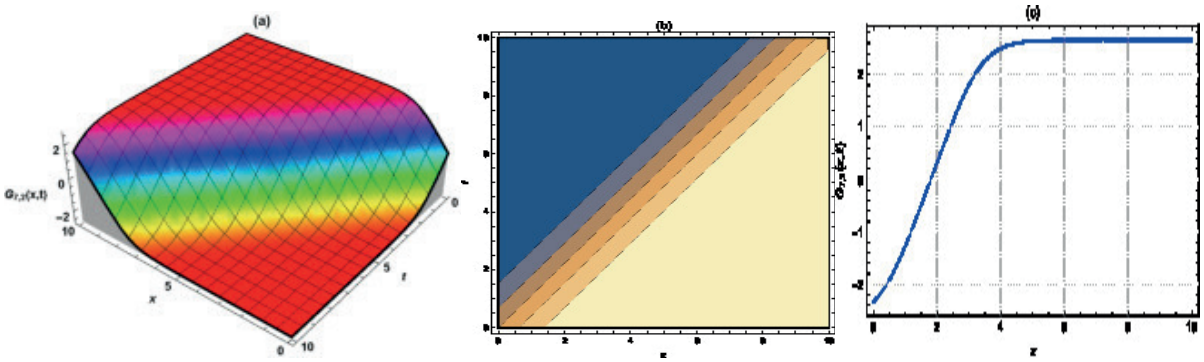

Figure 6: The numerical simulations corresponding to $|G_{7,2}|$ *given by Eq.(33), for* $m = 1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta = 0.1, v = 0.9, n = 0.2, h_4 = 0.24$.

If $m \to 0$, then the periodic wave solution is obtained

$$G_{7,3}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\tan(tv-x)}{\sqrt{n}\sqrt{-\frac{h_4v^2\left((\delta+2v^2)\tan^2(tv-x)+3v^2\right)}{v^4-\delta^2}}}, \quad (34)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}+2\right)\left(9-\left(4-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+2\right)\right).$$

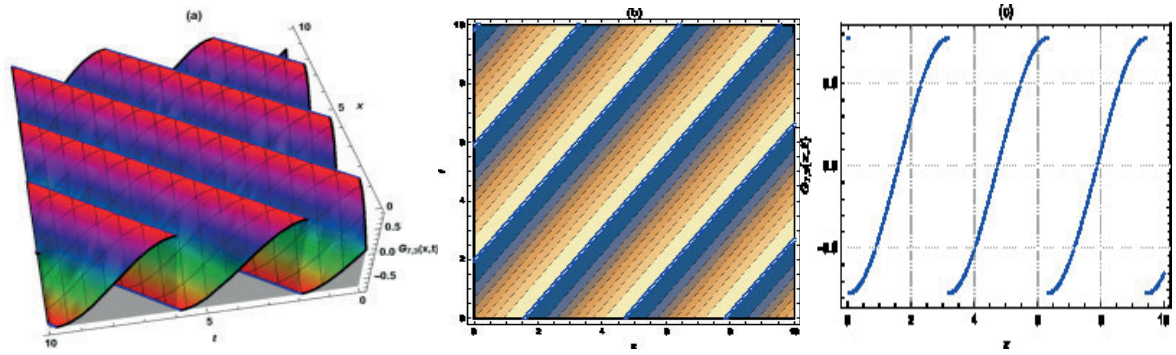

Figure 7: The numerical simulations corresponding to $|G_{7,3}|$ *given by Eq.(34), for* $m = 1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta = 0.5, v = 0.8, n = 0.3, h_4 = 0.06$.

8. If $l_0 = 1$, $l_2 = 2m^2 - 1$, $l_4 = -m^2(1-m^2)$, $0 < m < 1$, then $P(\zeta) = sd(\zeta, m)$ and we have

$$G_8(x,t) = \alpha_1\left(\frac{sd(\zeta,m)}{\sqrt{f(sd(\zeta,m))^2+g}}\right), \qquad (35)$$

where f and g are given by

$$f = -\frac{h_4v^2(\delta+(2m^2-1)v^2)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = -\frac{3h_4v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(2m^2+\frac{\delta}{v^2}-1\right)\left(-\left(2(2m^2-1)-\frac{\delta}{v^2}\right)\left(2m^2+\frac{\delta}{v^2}-1\right)-9(1-m^2)m^2\right)=0.$$

9. If $l_0 = 1-m^2$, $l_2 = 2-m^2$, $l_4 = 1$, $0<m<1$, then $P(\zeta) = cs(\zeta,m)$ and we have

$$G_{9,1}(x,t) = \alpha_1\left(\frac{cs(\zeta,m)}{\sqrt{f(cs(\zeta,m))^2+g}}\right), \qquad (36)$$

where f and g are given by

$$f = \frac{h_4v^2\left((m^2-2)v^2-\delta\right)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = \frac{3h_4(m^2-1)v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(-m^2+\frac{\delta}{v^2}+2\right)\left(9(1-m^2)-\left(2(2-m^2)-\frac{\delta}{v^2}\right)\left(-m^2+\frac{\delta}{v^2}+2\right)\right)=0.$$

If $m\to 1$, then the singular soliton solution is obtained

$$G_{9,2}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\operatorname{csch}(tv-x)}{\sqrt{n}\sqrt{-\frac{h_4v^2\operatorname{csch}^2(tv-x)}{v^2-\delta}}}, \qquad (37)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}+1\right)\left(-\left(2-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+1\right)\right).$$

If $m \to 0$, then the periodic wave solution is obtained

$$G_{9,3}(x,t) = -\frac{i\sqrt{2}\sqrt{h_4}v\cot(tv-x)}{\sqrt{n}\sqrt{-\frac{h_4v^2\left((\delta+2v^2)\cot^2(tv-x)+3v^2\right)}{v^4-\delta^2}}}, \quad (38)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}+2\right)\left(9-\left(4-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+2\right)\right)=0.$$

10. If $l_0 = -m^2(1-m^2)$, $l_2 = 2m^2-1$, $l_4 = 1$, $0 < m < 1$, then $P(\zeta) = ds(\zeta,m)$ and we have

$$G_{10}(x,t) = \alpha_1\left(\frac{ds(\zeta,m)}{\sqrt{f(ds(\zeta,m))^2+g}}\right), \quad (39)$$

where f and g are given by

$$f = -\frac{h_4v^2(\delta+(2m^2-1)v^2)}{(m^4-m^2+1)v^4-\delta^2},$$

$$g = -\frac{3h_4m^2(m^2-1)v^4}{(m^4-m^2+1)v^4-\delta^2},$$

under the constraint condition

$$h_4^2\left(2m^2+\frac{\delta}{v^2}-1\right)\left(-\left(2(2m^2-1)-\frac{\delta}{v^2}\right)\left(2m^2+\frac{\delta}{v^2}-1\right)-9(1-m^2)m^2\right)=0.$$

11. If $l_0 = \frac{1-m^2}{4}$, $l_2 = \frac{1+m^2}{2}$, $l_4 = \frac{1-m^2}{4}$, $0 < m < 1$, then $P(\zeta) = nc(\zeta,m) \pm sc(\zeta,m)$ or $P(\zeta) = \frac{cn(\zeta,m)}{1\pm sn(\zeta,m)}$ and we have

$$G_{11,0}(x,t) = \alpha_1\left(\frac{nc(\zeta,m)\pm sc(\zeta,m)}{\sqrt{f(nc(\zeta,m)\pm sc(\zeta,m))^2+g}}\right), \quad (40)$$

or

$$G_{11,1}(x,t) = \alpha_1 \left(\frac{\frac{cn(\zeta,m)}{1\pm sn(\zeta,m)}}{\sqrt{f\left(\frac{cn(\zeta,m)}{1\pm sn(\zeta,m)}\right)^2 + g}} \right), \quad (41)$$

where f and g are given by

$$f = -\frac{8h_4v^2(2\delta+(m^2+1)v^2)}{(m^4+14m^2+1)v^4-16\delta^2},$$

$$g = \frac{12h_4(m^2-1)v^4}{(m^4+14m^2+1)v^4-16\delta^2},$$

under the constraint condition

$$h_4^2\left(\frac{1}{2}(m^2+1)+\frac{\delta}{v^2}\right)\left(\frac{9}{16}(1-m^2)^2-\left(m^2-\frac{\delta}{v^2}+1\right)\left(\frac{1}{2}(m^2+1)+\frac{\delta}{v^2}\right)\right)=0.$$

If $m \to 1$, then the exponential solution is obtained

$$G_{11,2}(x,t) = \frac{i\sqrt{h_4}ve^{x-tv}}{\sqrt{n}\sqrt{-\frac{h_4v^2e^{2x-2tv}}{2v^2-2\delta}}}, \quad (42)$$

such that

$$h_4^2\left(\frac{\delta}{v^2}+1\right)\left(-\left(2-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+1\right)\right)=0.$$

If $m \to 0$, then the combined periodic wave solutions are retrieved

$$G_{11,3}(x,t) = \frac{i\sqrt{h_4}v(\sec(tv-x)-\tan(tv-x))}{\sqrt{2}\sqrt{n}\sqrt{-\frac{h_4v^2(4\delta+(v^2-4\delta)\sin(tv-x)+5v^2)}{(v^4-16\delta^2)(\sin(tv-x)+1)}}}, \quad (43)$$

or

$$G_{11,4}(x,t) = -\frac{i\sqrt{h_4}v\cos(tv-x)}{\sqrt{2}\sqrt{n}(\sin(tv-x)-1)\sqrt{-\frac{h_4v^2(2(2\delta+v^2)\cos^2(tv-x)+3v^2(\sin(tv-x)-1)^2)}{(v^4-16\delta^2)(\sin(tv-x)-1)^2}}}, \quad (44)$$

are obtained, such that

$$h_4^2\left(\frac{\delta}{v^2}+\frac{1}{2}\right)\left(\frac{9}{16}-\left(1-\frac{\delta}{v^2}\right)\left(\frac{\delta}{v^2}+\frac{1}{2}\right)\right)=0.$$

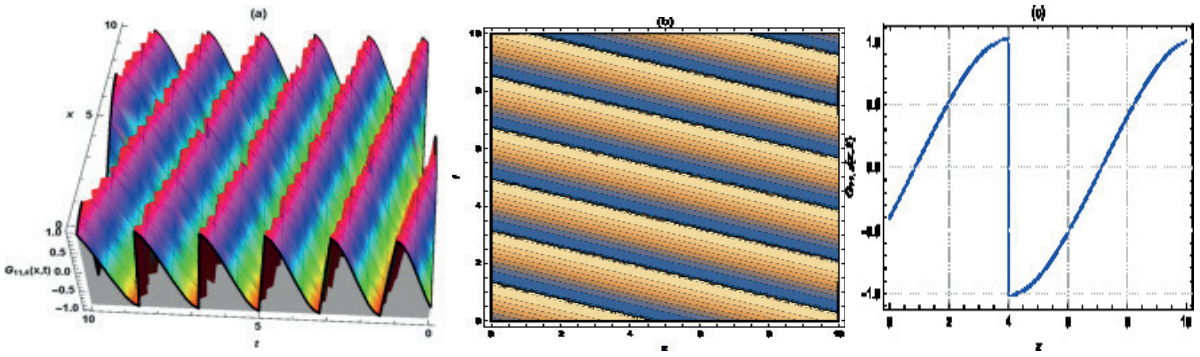

Figure 8: The numerical simulations corresponding to $|G_{11,4}|$ *given by Eq.(44), for* $m=1$ *; (a) is the 3D graphic, (b) is the 2D-contour graphic while (c) is the 2D graphic for* $\delta=1.9, v=-3.5, n=1.4, h_4=0.3$.

Interpretation and Discussion of the Results

The movement patterns of the generated solutions are shown by plots of three-dimensional surfaces. It is straightforward to notice differences in 3D surface images when using certain graphs, density plots and contours. As a result, contoured and density graphs are displayed for each 3D object.

Our goal is to show the significance of the constructed outcomes and the paper's achievements. We are able to reach our desired goal by comparing our findings to those that have lately been published [18-21]. The following techniques have been used by researchers to retrieve solitons to the proposed nonlinear Duffing equation, Aminah used the sine-cosine function method [18] to construct the periodic wave solutions. The exact particular solutions are obtained including periodic solutions using the $\left(\frac{\Phi(\xi)}{2}\right)$ −expansion method [19] by Jalil et al. Balaji solved the Duffing equation for integral and non-integral forcing terms [20]. The first integral method [21]. However, by applying specific unique values for the aforementioned parameters, it can be seen that almost all of our solutions are completely different from those found in [18-21].

Conclusions

In this paper, the nonlinear Duffing equation is studied. Bright, kink, periodic, and combined periodic soliton solutions are explicitly extracted using the $\varphi 6$-model expansion method. Additionally, singular soliton solutions are seen positively. Figures $1-8$ depict the soliton solutions at any given moment, which is significant for the transfer of energy from one place to another. For different parameter values, it is the traveling wave's internal dynamics. We may get the conclusion that for varying values of each, the traveling wave behavior changes. The findings of this study are expected to contribute to strengthening the nonlinear dynamical properties of the equation. A practical and efficient approach to solving a wide range of nonlinear partial differential equations is suggested by the method.

Declarations

Ethical Approval

Not applicable as there is no research using either human or animal subjects on this topic

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Polymeric Ionic Liquid (PIL) as Solid Polymer Electrolyte; A Mini Review

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Tarik Eren[2]

Abstract

Due to their numerous inherent characteristics, such as superior mechanical and chemical stability, structural controllability over the IL species and macromolecular backbone, and leak-proof nature and thereby improved safety, polymeric ionic liquids that contain both ionic liquid-like moieties and polymer frameworks are emerging as alternative electrolyte/binder candidates for Li-based rechargeable batteries. In this paper we review polymeric ionic liquid that been used as polymer electrolyte. With an emphasis on the structural-property correlations, current developments, and improvements on the usage of PIL-based electrolytes and binders for Li-based rechargeable batteries are reviewed and discussed. From the perspective of the chemistry of Li-based batteries, future directions, and enhancements of the characteristics of PIL-based materials are provided.

Keywords: Polymeric ionic liquid; polymer electrolyte; lithium ion batteries

Introduction

The term "lithium-ion battery" refers to a wide range of battery chemistries, form factors, sizes, and cell architectures. The positive electrode (cathode), the negative electrode (anode), and the separator are the three functional layers of a lithium-ion battery cell. A polymeric membrane saturated with a liquid electrolyte serves as the separator, allowing lithium-ion transit while preventing direct contact between the electrodes. To maximize the effective surface area available for energy storage, these thin layers are rolled or layered, and then packaged in an outer cell housing. By sandwiching an electrolyte between a lithium metal (anode) and a

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composite cathode, a lithium-ion battery can be simply manufactured.

The basic principle and function of a rechargeable lithium-ion battery are depicted in Figure 1. Between the two electrodes is an ion-conducting electrolyte (containing a dissociated lithium conducting salt). The separator, which is a porous membrane that electrically separates the two electrodes, is also in this position. During charging and discharging, single lithium ions travel back and forth between the electrodes of lithium-ion batteries and are intercalated into the active materials. Electrons are released when discharging, for example, when lithium is deintercalated from the negative electrode (copper acts as a current collector). Mixed oxides, for example, are active materials in the positive electrode Graphite and amorphous carbon compounds are the most common negative electrode materials. Active materials, such as mixed oxides, are used in the positive electrode. The negative electrode's active components are primarily graphite and amorphous carbon compounds. These are the materials that have lithium intercalated into them. During discharging, lithium ions migrate from the negative electrode to the positive electrode via the electrolyte and separator, as shown in Figure 1. At the same time, electrons as electricity carriers migrate from the negative electrode to the positive electrode via an exterior electrical connection (cable) (aluminum as current collector). During charging, the process is reversed, with lithium ions migrating from the positive electrode to the negative electrode via the electrolyte and separator.

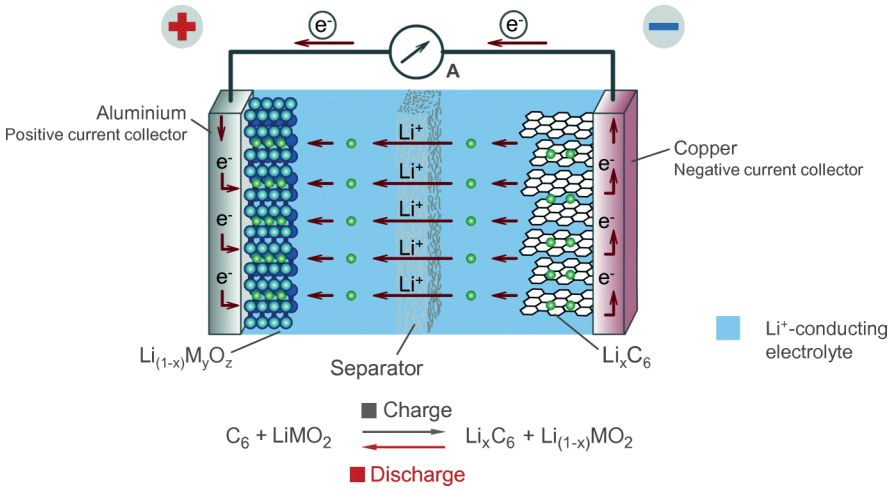

Figure 1: Configuration of a lithium-ion battery (shown is the discharging process). Reproduce from ref [1].

One of the most important components of LIBs is electrolyte. They must not only assure quick ion transport and satisfactory chemical and electrochemical stability, but also reduce the safety risk posed by thermal instability, flammability, and the likelihood of leakage. Unfortunately, traditional flammable and corrosive organic liquid electrolytes, have severe safety concerns when it comes to commercialization and scaling up of lithium metal batteries.

In addition, the inability of liquid electrolyte to prevent lithium dendrite formation and growth, which can lead to a battery short circuit, is a significant impediment to the commercialization and scaling up of lithium metal batteries.

Nowadays consumer electronics, cars, large-scale energy storage, and integrated power systems, as well as telecommunication equipment and applications, all use lithium-based rechargeable batteries) due to its high density energy and excellent cycling life [2]. Despite the fact that typical carbonate-based liquid electrolytes have a high ionic conductivity at room temperature, the organic solvent included in them poses a risk of leakage and combustion, posing serious safety concerns. Solid-state electrolytes, both inorganic and solid polymer electrolytes (SPE), can help to solve these problems [3].

SPE stands for solid-state electrolyte, which consists of salt(s) distributed in a polymer matrix. Due to its enormous potential in substituting liquid electrolytes in electrochemical devices, SPE became the subject of research studies [4]. However, due to their high interfacial resistance and low bulk conductivity (10^{-7} S/cm) at ambient temperatures, SPEs have not yet reached practical accessibility [5]. As a result of these discoveries, numerous modifications have been performed by various research groups with the goal of enhancing the mechanical, electrochemical, and thermal durability of polymer electrolytes. To achieve this, the polymer's amorphous form and low transition temperature (Tg) are important qualities because this state allows the polymer chain to move more freely, boosting ion mobility. Poly(ethylene oxide) (PEO), poly(acrylonitrile) (PAN), poly(methylmetacrylate) (PMMA), poly(vinylidene fluoride) (PVF), and other polymers have been explored and employed as the matrix for this purpose [6]. In essence, polymeric ionic liquid is a suitable contender to be the host polymer for polymer electrolytes because of their amazing performance [7].

Polymeric ionic liquids (PILs), also known as polymerized ionic liquids, are a type of polyelectrolyte that has an ionic liquid (IL) species in each monomer repeating unit, which is

linked together by a polymeric backbone to form a macromolecular structure. PILs combine some of the unique properties of ILs in the polymer chain, resulting in a new class of polymeric materials. Rapid advances in the chemistry and physics of polyelectrolytes have resulted in the development of unique and versatile polyelectrolytes that are important for basic research and provide materials for new solutions in a variety of systems. PILs research is currently in a protracted expansion period Figure 2, with several unique properties and applications being discovered recently.

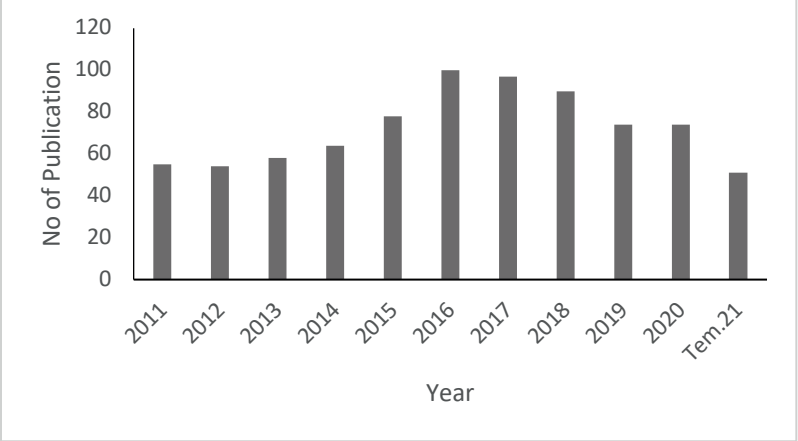

Figure 2: Plot of number of publication vs year of publication obtained from Scopus by searching for topics 'poly ionic liquids'.

PILs have great potential as neoteric electrolyte in battery design. Due to the pressing safety problem concerning solvents as industry pursues batteries with smaller volume and higher power density, several academics are researching ILs and PILs as electrolytes instead of traditionally utilized solvent-based electrolytes. Additionally, in battery fabrication there are two main safety concern rise on which are flammability of organic solvents used in electrolytes and dendrite generation from electrodes (in lithium batteries) .

PILs are becoming more popular as a polymer component in polymer gels or ion gels, which contain an organic solvent or an ionic liquid electrolyte, respectively is incorporated into the PILs. Although they have been successfully shown as polymer electrolytes for energy storage and conversion devices, these gels typically have a low salt content, which results in a low transference number . Additionally, polymerization of ionic liquid (IL) monomers shown

greater mechanical integrity than their ILs analogs but often results in lower ionic conductivities and a greater Tg due to cation immobilization. Various PIL structures, such as singly charged polymers, zwitterionic polymers, polyelectrolyte blends, and ion-containing block copolymers, are under investigation in the current literature. Researchers have concentrated on two distinct but equally effective techniques to solving the paradox between mechanical integrity and ion conductivities during their efforts to solve the problem:

i) Increase ILs' mechanical integrity

ii) Increase PILs' ion conductivities

Properties of PILs

Polymers formed from monomeric ionic liquids are known as poly (ionic liquids) (PILs). PILs exhibit excellent mechanical and ion conductivity properties, paving the path for more compact and safer solid electrolytes. PILs can have customizable mechanical properties, morphologies, and multi-responsive behavior, thanks to the rational design of their chemical compounds and architectures [11]. PILs, on the other hand, have lower ion conductivities than ILs due to restricted segmental mobility.

In PILs, it's vital to note that confined ions contribute very little to ion conductivity, and that "free" counter ions control ion conductivity to a great extent. Glass transition temperature (Tg) in PILs is generally inversely proportional to ion conductivities, much like viscosity in ILs [8]. Lower Tg corresponds to more mobile counter-ion matrices, resulting in higher ion conductivities. The lowering of Tg is aided by two key mechanisms. In terms of free volume, bulkier ions introduce more free volume, lowering Tg. Ions act as pendant groups in this scenario, and studies have shown that larger pendant groups tend to lower Tg by introducing more free volume. Ions serve as pendant groups in this scenario, and research has shown that larger pendant groups reduce Tg by introducing more free space [9]. Electrostatic interactions, which operate as physical crosslinks in polymer matrices to hinder mass transfer, are another consideration. As a result of the weak electrostatic interactions, physical crosslinks are weak and Tg is low [8]. Nevertheless, low Tg isn't the only criterion for increased conductivities, though. With little change in Tg, little structural variation can improve ion conductivity by reducing electrostatic interactions. In this sense, P-based PILs held tremendous promise for

enhancing ion conductivity over their N-based counterparts while sacrificing mechanical qualities (lower Tg).

The use of solid electrolyte instead of liquid electrolytes is predicted to solve battery safety issues, reduce electrolyte/electrode interface side reactions, permit the use of lithium metal anodes, and extend battery life. Flexibility, processability, and electrode adherence are all advantages of solid polymer electrolytes. Most PIL-based electrolytes that have been published have been focused on increasing total ionic conductivity, with the expectation that Li-ion conductivity will follow. The chemical structures of PILs that reported in previous literature are shown in Figure 3.

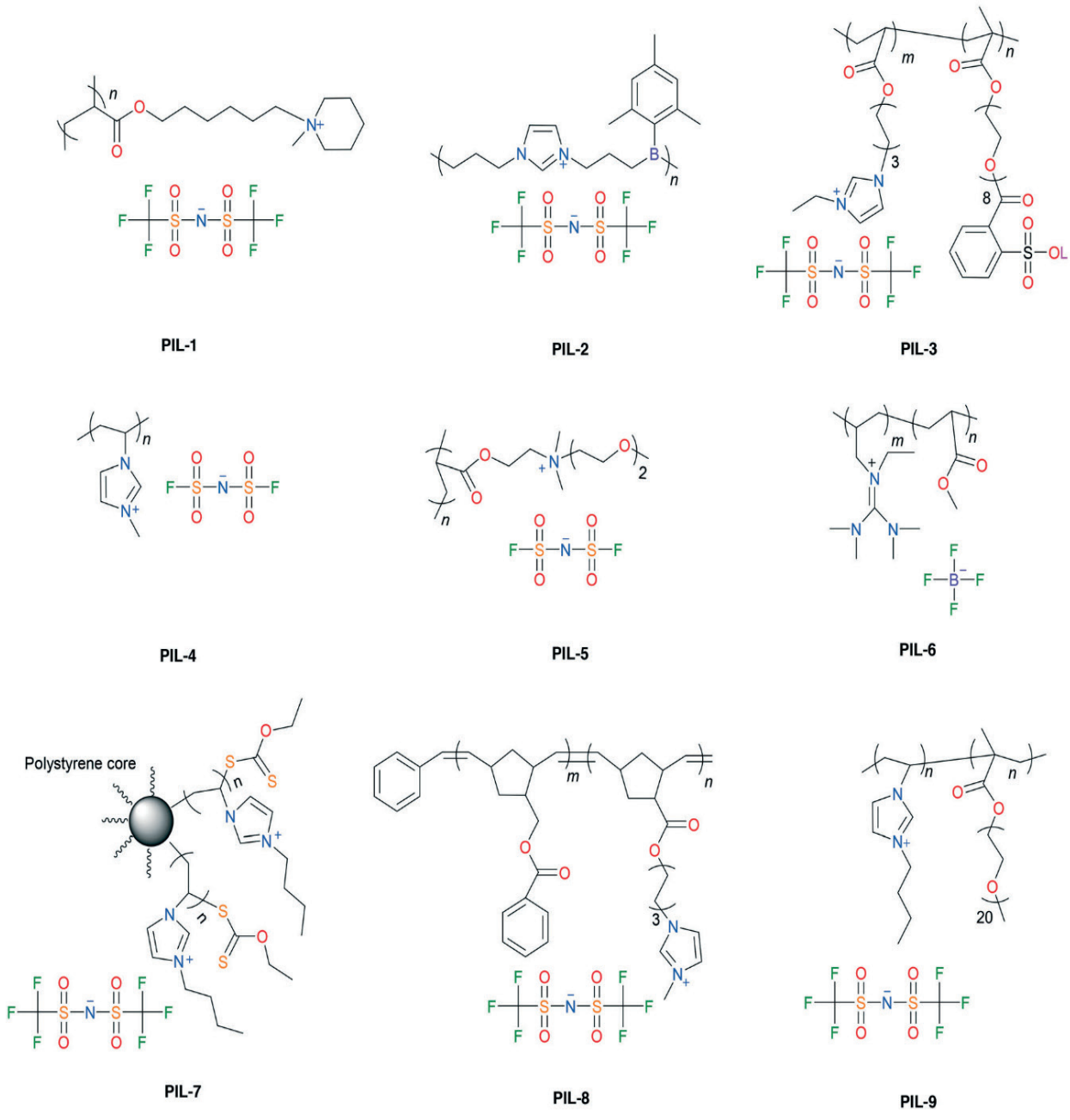

Figure 3: Chemical structure of PILs

Ohno et al. [10] conducted a thorough investigation into the structure-ionic conductivity correlations of the PIL-based Li-ion conducting SPEs in the 2000s. They considered factors like the type of cation and anion in PILs, the distance between the polymer backbone and ionic center, the concentration of lithium salt, etc. The molecular design of PIL-based SPEs might be inferred from a number of significant hints, using the PIL with piperidinium cations as an example (PIL-1 in Figure 3). 1) The ionic center is separated from the rest of the material by

six CH_2 units, which helps to achieve high ionic conductivity because a longer spacer would have little effect on the Tg of PILs but would result in a significant drop in the concentration of ions, which would lower conductivity; and 2) $TFSI^-$ anion, which functions as a plasticizer for PILs and has a flexible sulfonimide core ($-SO_2^-$, NO^-,SO^{2-}), reduces the rise in Tg following the polymerization of the ionic liquid monomers; 3) the stability of PIL-based SPEs is improved by the piperidinium cation, which is electrochemically more stable than the imidazolium cation.

The Li-ion transference number for the piperidinium cation-based SPEs was larger (0.43 at 25 °C) than that of the imidazolium cation-based SPE, which was around 0.11 at 25°C; the mechanism is still not completely understood. In contrast, Ohno et al. used two ways to further increase the Li-ion transference number:

1) adding a specific anion receptor (such as alkylborane) to the polymer framework of the polymer backbone [such as PIL-2 in Figure 3] $\sigma = 1.9 \times 10^{-5}$ Scm^{-1} (50 °C), $T_{Li^+} = 0.87$ (30 °C)][11].

2) copolymerizing the lithium salt-containing monomer (like PIL-3 in Figure 3) with the ionic liquid monomer ($\sigma = 1.0 \times 10^{-5}$ Scm^{-1} and $T_{Li^+} = 0.5$ at 30 °C) [12].

In addition to those TFSI-based PILs, Zhou et al.[13] presented a number of polycation type PILs employing FSI (PIL-4 and PIL-5 in Figure 3), an analog of TFSI but with a smaller size and higher interfacial compatibility with different electrodes as demonstrated in the studies of IL-based electrolytes. Because FSI has a greater degree of conformational freedom than TFSI, it exhibits lower glass transitions than TFSI-based PILs, leading to higher ionic conductivities [14]. It is noteworthy that the FSI-based PIL electrolytes shown good chemical stability against the lithium metal (Li°) electrode. This may be because, as recently demonstrated in Li-S cells using the LiFSI/PEO electrolyte, the reduction of FSI results in the creation of a LiF-rich SEI layer [15].

Additionally, the functionalization of the ammonium cation with adaptable alkylene-ether groups boosted the mechanical flexibility and ionic conductivity of the PIL-based electrolytes by lowering the Tg of PILs (Figure 4 a and b).

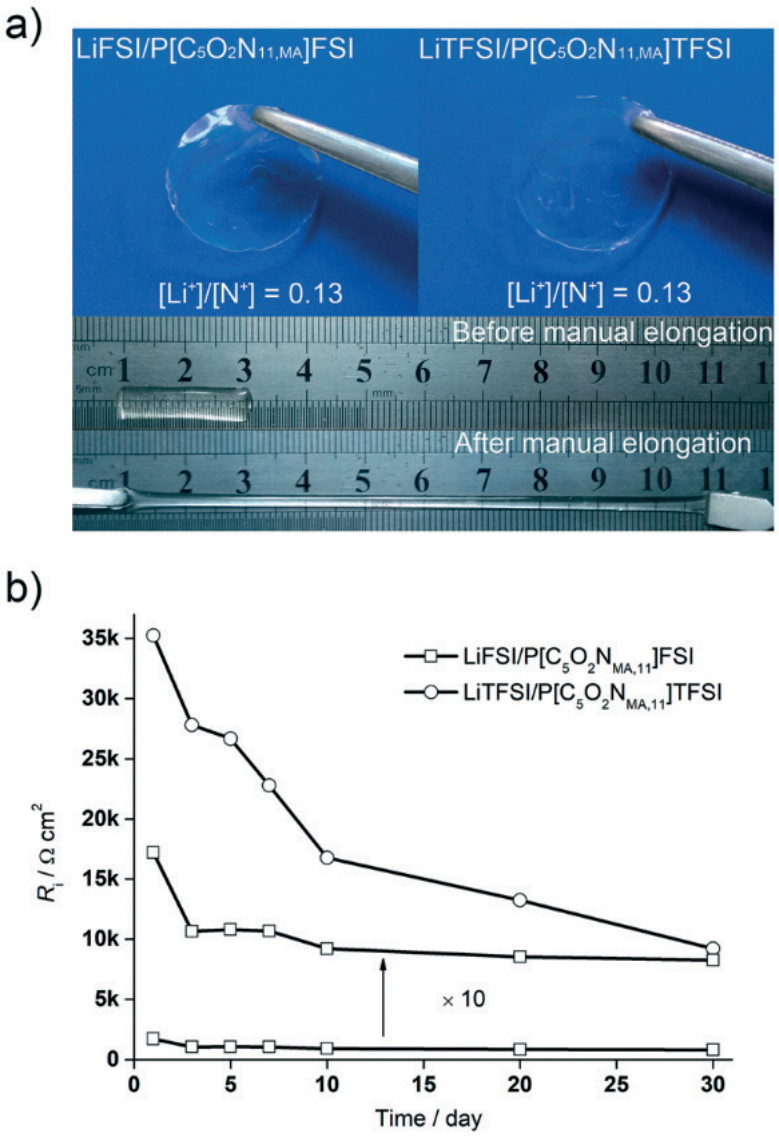

Figure 4: a) A photograph of the polymer electrolyte membrane as it was made using polyIJN,N-dimethyl-N-[2-(methacryloyloxy) ethyl]. Poly(IJN,N-dimethyl-N-[2-(methacryloyloxy)ethyl] and N-[2-(2-methoxyethoxy)ethyl]ammonium bis(IJfluorosulfonyl)imide (PijC5-O2N11,MA]FSI, PIL-5 in Scheme 1) PijC5O2N11,MA]TFSI is the chemical name for N-[2-(2-methoxyethoxy) ethyl]ammonium bis(trifluoromethanesulfonyl)imide. b) The interfacial resistance (Ri) of Li/SPEs with time as a function of storage time at 60 °C. Please take note that the LiFSI/PIL-5 electrolyte values have been 10 times upshifted for clarity. Reproduced from reference [13] with permission. 2015 Elsevier Copyright.

Yang et al.99 examined a variety of guanidinium-based PILs (PIL-6 in Figure 3) in addition to PILs containing imidazolium, ammonium, pyrrolidinium, and piperidinium cations because guanidinium-based discrete molecule ILs had previously been found to have good

electrochemical properties. At 30 °C, the ionic conductivity of the PIL electrolytes based on guanidinium and containing the tetrafluoroborate anion (BF_4) attained a comparatively high value of 1.3 x 10^{-4} Scm^{-1}. These findings strongly imply that the expertise accumulated in IL-based electrolytes may be passed down and effectively utilized to swiftly identify and screen acceptable PIL-based electrolytes for Li-based batteries [16].

Controlling and fine-tuning the architecture of PIL matrices is now possible thanks to the development of polymerization techniques. Using a combination of atom transfer radical self-condensing vinyl polymerization (ATR-SCVP) and reversible addition-fragmentation chain transfer polymerization (RAFT), Zhang et al. described a hyperbranched star PIL (PIL-8, Figure 3) with a polystyrene core and imidazolium-based ionic arms [17]. He and colleagues 101 created norbornene-based PILs with imidazolium cations using Grubb's catalyst in ring-opening metathesis polymerization. These PIL-based electrolytes shown good mechanical strength and thermal stability while having a relatively low ionic conductivity of around 10^{-5} Scm^{-1} at ambient temperature [18].

PIL-based SPEs have rarely been used in Li-based cells due to the high interfacial resistance and low ionic conductivity of the Li° electrode. As a polymer matrix for Li° || LFP ($LiFePO_4$) cells, Zhang et al.[19] reported using a copolymerized PIL comprising imidazolium cations and flexible ethylene oxide units (PIL-9). At 60 °C, the LiTFSI/PIL-9 electrolyte had an approximate 104 S cm1 ionic conductivity, and the matching Li° LFP cell performed well in terms of cycle and rate. The comparison between a non-ionic polymer matrix and PIL-9 in particular revealed that the ionic segment in the PIL efficiently inhibited the growth of lithium dendrites and, as a result, enhanced the interfacial behavior between the electrolyte and Li° electrode PIL-based

The electrochemical characteristics of Li-based cells utilizing PIL-based electrolytes are summarized in Table 1 below.

Table 1: Li-based cells' electrochemical performance with PIL-based electrolytes

No	Electrolyte	σ/S cm^{-1}	Cell type	Cell performance	T_{cycle}	Ref
1	LiTFSI/PIL-9	4.0×10^{-4} at 60 °C	Li° ‖ LFP	130 mA h g−1 at C/10 after 100 cycles	60	[20]
2	LiTFSI/PY_{14}TFSI/PIL-10	1.6 X 10^{-4} at 40 °C	Li° ‖ LFP	140 and 50 mA h g^{-1} at C/5 and C/2, respectively, with good capacity retention	40	[21]
3	LiTFSI/SN/PIL-10	5.7 x 10^{-4} at 25 °C	Li° ‖ LFP	150, 132, and 122 mA h g^{-1} at C/10, C/2, and 1C, respectively, good capacity retention for 40 cycles	25	[22]
4	LiTFSI/PY12FSI/PIL-10	1.5 X 10^{-4} at 25 °C	Li° ‖ LFP	122 mA h g−1 at C/5 for the 1st cycle and gradually increased to 150 mA h g^{-1} after 150 cycles	25	[23]
5.	LiTFSI/PY12FSI/PIL-10	3.3 X 10^{-4} at 40 °C	Li° ‖ LFP	150 mA h g^{-1} at C/5 for 150 cycles without obvious capacity fading	40	[23]
6.	LiTFSI/EMITFSI/PIL-10	3.4×10^{-3} at 25 °C	Li° ‖ LFP	160 mA h g^{-1} at C/5 and 20 mA h g^{-1} at 5C	22	[24]
7.	LiTFSI/PEG800/PIL-10	9.3×10^{-5} at 80 °C	Li° ‖ LFP	140 mA h g−1 at C/5 after 70 cycles	80	[25]
8.	LiTFSI/PY1.1O2TFSI/TEOS/PIL-10	5.1×10^{-4} at 25 °C	Li° ‖ LFP	138 mA h g−1 at C/2 and 120 mA h g^{-1} at 1C	25	[26]
9.	LiTFSI/EMITFSI/C1-4TFSI/PIL-10	1.6 x 10^{-3} at 25 °C	Li° ‖ LFP	150 mA h g−1 at C/10 for 100 cycles without obvious capacity fading	25	[27]

Ammonium Vs Phosphonium Based Poly İonic Liquid

In recent, nitrogen-based (N-based) salts,(including imidazolium [14], pyridinium [15], 1,2,3-triazolium [16], ammonium [17]) , and phosphonium-based salts [18], were used in the

development of innovative ILs and PILs electrolytes. The atomic radii and electronegativity of nitrogen and phosphorus atoms differ greatly, resulting in distinct cationic architectures. The atomic weight of nitrogen is 14.01 g/mol, while the atomic weight of phosphorus is 30.97 g/mol. Hence, the atomic radius of phosphorus is bigger than nitrogen and differences in electronegativity lead to a varied charge distribution for the corresponding cation [19].

On the other hand, Wang et al. [12] used ab initio calculations to estimate the specific charges on each atom for the ammonium and phosphonium cations. Due nitrogen is more electronegative than carbon, the nitrogen in ammonium cations had a little negative partial charge of -0.5 eV, while the surrounding alpha carbons had a slightly positive charge of 0.3 eV. Conversely, because phosphorus is less electronegative than carbon, phosphonium cations have a reversed charge density. Thus, the phosphorus atom has a partial positive charge of 1.1 eV, whereas the carbon atoms around it have a partial negative charge of -0.2 eV. These discrepancies are predicted to have a significant impact on characteristics in new applications.

In comparison to their N-based counterparts, P-based ILs and PILs exhibit greater thermal stability, base stabilit, and ion conductivities [28][29][30]. For instance, Poly(4-Vinylbenzyl Ammonium) and Poly(4-Vinylbenzyl Phosphonium) homopolymers with different alkyl replacements were examined by Long et al. P-based PILs shown an increase in the onset of thermal deterioration of over 100 °C [28]. Hoffman elimination and/or reversible Menschutkin degradation occur in ammonium salts (for benzylic protons). In contrast, phosphonium salts have higher thermal stability because they are less susceptible to both breakdown processes [28][17]. In comparison to their ammonium counterparts, phosphonium PILs also displayed higher ion conductivities (normalized with Tg). Al-kaline anion exchange membranes made of polyethylene functionalized with phosphonium pendant groups were created, according to Coates and colleagues[30].

In short, P-based ILs and PILs show great promise as novel electrolyte materials, although they have not received as much research as their ammonium analogs. Although ILs and PILs have numerous applications in gene delivery [31], antimicrobial coatings [32], gas separation [33], and conductive materials [34].

Phosphonium-Based Poly (İonic Liquid)S.

Due to negligible Hoffman removal and reverse Menschutkin degradation, P-based PILs demonstrated greater thermal stability than their N-based counterparts [28]. Long et al. created two series of styrenic PILs, as shown in Figure 5a, using either quaternary ammonium or phosphonium cations, and they discovered that P-based PILs had much higher thermal breakdown temperatures [28]. When compared to their N-based analogs, P-based PILs showed similar Tgs (Figure 5b) but significantly higher ion conductivities (Figure 5c), suggesting that they would make ideal electrolytes or ion exchange membranes with strong mechanical integrity and high ion conductivities.

Although there isn't a significant enough variation in size between nitrogen and phosphorus to noticeably alter the Tg, P-based PILs have weaker electrostatic interactions than N-based PILs, which leads to higher ion conductivities. The electronegativities of nitrogen, phosphorus, and carbon are the cause of the different electrostatic interactions between N-based PILs and P-based PILs [8]. Nitrogen has a partial negative charge in ammonium cations because it has a stronger electro- negativity than α – carbons. Positively charged α – carbons and counter anions are the main components of the electrostatic interaction. In contrast, because it has a lower electronegativity than carbon and produces slightly negatively charged -carbons, phosphorus has a partial positive charge in phos- phonium cations. The positive charge on phosphorus is effectively stymied by these negatively charged α – carbons, which also reduce electrostatic interactions. When substituting nitrogen with phosphorus, other researchers have shown a similar rise in ion conductivities [8]

Variations in alkyl substituent length, unlike the substitution of phosphorus for nitrogen, result in significant changes in Tg. Tg decrease from 91 °C to 68 °C (as shown in Figure 5b) when methyl R groups (PTMP) were swapped out for ethyl R groups (PTEP). The temperature at which the alkyl substituents continue to elongate in P-based PILs changes very little (68 °C, 71 °C, and 66 °C for ethyl, propyl, and butyl, respectively). With the exception of methyl substituted polymers, shorter alkyl substituents displayed higher ion conductivities at the same temperature (Figure 5c), likely because their Tg was higher than that of the other PILs by 20 °C.

After adjusting for Tg, the ion conductivities consistently followed the predicted pattern: methyl > ethyl > propyl > butyl (Fig. 5d). This tendency is consistent with ILs, which correlate smaller

size and fewer non-ionic content with increased mobility and ion content, respectively. This tendency explains higher mobility and ion content from smaller size and reduced non-ionic content, respectively, and is consistent with ILs. Gin, Noble, and colleagues investigated the identical P-based PILs with longer alkyl substituents (hexyl and octyl), and they noticed a steady decrease in Tg [35]. The ion conductivities of hexyl and octyl substituents both showed the same pattern at high temperatures: shorter substituents showed greater conductivity. The difference in ion conductivity between long alkyl substituents was still present, although it was less pronounced. Even after Tg normalization, P-based PILs still had greater ion conductivities than their N-based counterparts, which further proved the superiority of P-based PILs in terms of conductivity.

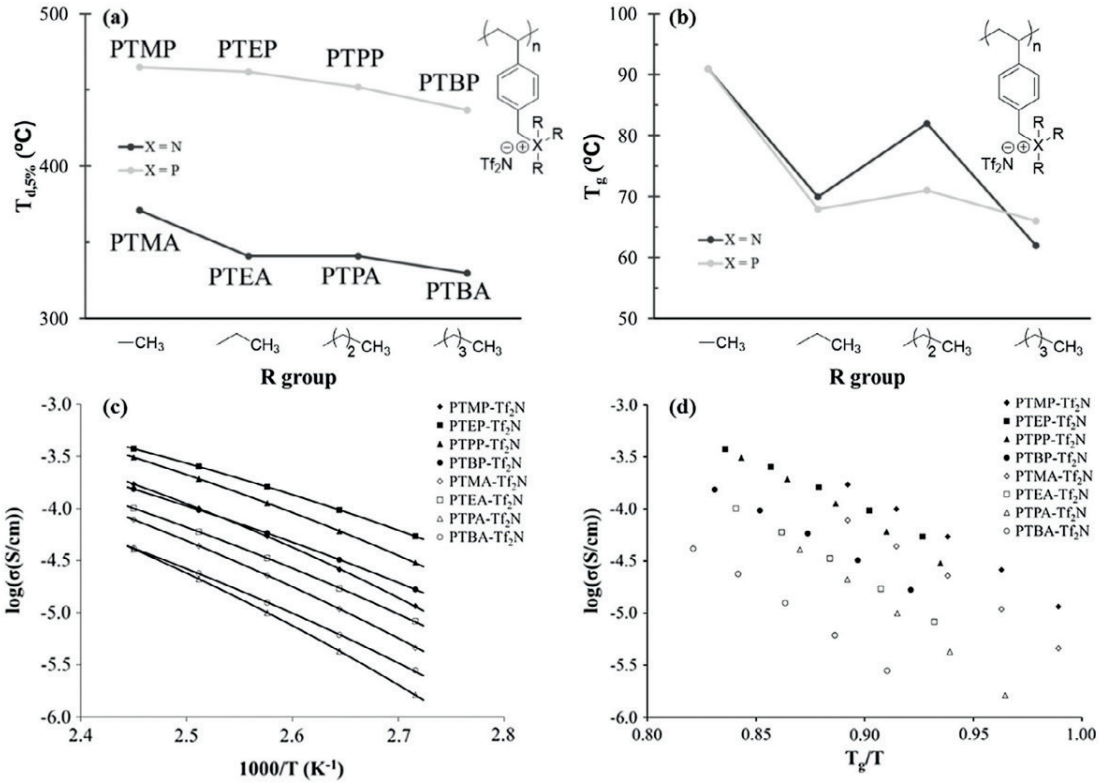

Figure 5. Comparison of P-based PILs and N-based PILs' thermal stability (a), Tg (b), temperature-dependent ion conductivities (c) , and ion conductivities normalized with Tg (d). Reproduced with permission from ref [36].

Future Direction

PIL-based materials chemistry has entered a highly interesting period because of the continual

discovery of new PIL chemical structures. Chemists have progressed beyond the synthesis and structural characterization of new PILs, and further work will be done to integrate PILs into usable materials that address problems and difficulties in areas such as life science, energy, and the environment.

In this mini review, we described the difference of two type PILs including nitrogen based and phosphonium based. It shows that phosphonium based PILs has an advantage regarding electrical properties compared to nitrogen based. Phosphonium-based PILs, on the other hand, have an intriguing potential as solid/gel state electrolytes, such as ion gels [2]. Hence, Phosphonium based PILs with microphase separated morphologies, programmable and responsive mechanical properties, and continuous ion conductivity improvement have a promising future in innovative electrolyte applications.

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Nanotechnology Agents: What are their Effects on Cancer Cells? How Do they Inhibit Cancer?

Souandaou Athoumani Ali[1]

Abstract

Nanotechnology is an engineering, and technology science used in different fields in order to produce new and higher-quality products. Due to the selectivity and the ability of this science, it has been used to increase the potential of nanotechnology as a new research area in many fields such as chemistry, physics, and biology in recent years. The application of nanoparticles in cancer therapy has remarkably increased the therapeutic efficacy while decreasing side effects on normal tissues. In addition, nanoparticles can be used in combination with cancer standard treatment to support and increase the effects of chemotherapeutic drugs. In recent years, the properties of nanoparticles such as targeting cancer cells, delivering and releasing drugs in a regulated manner, and detecting cancer cells with enormous specificity and sensitivity have attracted the intention of researchers to nanotechnology. In this review, we will attempt to summarize the important use of nanoparticles in cancer treatment and the main targets and therapeutic possibilities pathways of nanoparticles in cancer therapy. Understanding the main mechanism of nanoparticles in cancer will make important contributions to cancer, especially in the development of effective anticancer agents and in clinics.

Keywords: Nanotechnology, Nanoparticles, Cancer, therapeutic effects

Introduction

Cancer is one of the leading causes of death worldwide and is considered a major economic barrier to increasing life expectancy. Due to the increase in cancer cases, cancer has become a major health problem for decades. According to studies, it is estimated 19.3 million new cancer cases and 10.0 million cancer deaths worldwide in 2020 [1]. Cancer incidence and death rate

increase by approximately 1% each year, and this increase is expected to rise to 26.4 million new cancer cases and 1.7 million cancer deaths by 2030 [2]. The main goal of most cancer treatment strategies is to achieve the desired therapeutic agent concentration at tumor sites, thereby minimizing damage to normal cells while destroying cancerous cells. With this vision, it is necessary to develop agents with high potential that will make an essential contribution to the prevention, detection, and treatment of cancer. In this context, we should focus on developing a variety of targeted therapeutic strategies and drugs to overcome the problems associated with conventional chemotherapeutic drugs so that cancer cells can be easily destroyed and clinically help to reduce cancer mortality [3] [5] [7].

Recent studies have shown that nanotechnologies are important and very effective in the prevention, diagnosis, and treatment of diseases [7]. Nanotechnology strategies in cancer treatment will outperform standard treatments [5]. This success can be further enhanced by the more widespread implementation of existing vaccine technologies, as well as the use of other technologies besides nanotechnology to increase the effectiveness of vaccination [4] [5]. The purpose of this review is to summarize the important use of nanoparticles in cancer therapy and the main targets and therapeutic possibilities pathways of nanoparticles in cancer therapy.

Nanotechnology as an Opportunity for Improve Cancer Therapy

Nanotechnology as a word means ‘engineer materials’. The term nanotechnology was first used at the University of Tokyo in 1974 by Norio Taniguchi to refer to the ability to design materials, particularly at the scale of nanometers. Nanotechnology is defined as the design and manufacture of materials, devices, and systems in nanometer dimensions. Therefore the essence of nanotechnology is size and control [6]. Cancer nanotechnology is emerging as a new research area encompassing biology, chemistry, engineering, and medicine and is expected to lead to major advances in cancer detection, screening, diagnosis, and treatment (Figure 1) [7]. The idea of designing Nano-scale substances to produce more effective cancer treatments is a panacea for the preferential eradication of cancer cells without seriously harming healthy cells. Nanotechnology is a multidisciplinary field that has emerged recently as one of the most suitable fields in cancer treatment [8]. Nano-medicine is the most viable application of nanoparticles and has incredible potential to revolutionize cancer treatments and diagnostics by developing nanoparticles for drug delivery purposes [9] [10]. In recent years, some

nanotechnology (NA) agents have been approved by the FDA (therapeutic nanocarriers-liposomes and albumin nanoparticles) and are allowed for clinical use. Of these, liposomal doxorubicin and albumin-bound paclitaxel (Abraxane1) can be given as examples. These nanoparticles or nanovectors have been developed for breast cancer chemotherapy [11] [14].

These Nanotechnological agents have four unique properties that distinguish them from other cancer therapeutics: (1) may themselves have therapeutic or diagnostic properties and be designed to carry a large therapeutic "payload"; (2) can bind to multivalent targeting ligands providing high affinity and specificity for target cells; (3) can be made to accommodate multiple drug molecules simultaneously enabling combinatorial cancer therapy and, (4) can bypass traditional mechanisms of drug resistance [8][12]. In addition, compared to other therapeutic agents, they are very effective in the clinic due to their properties: (i) their stability and time in circulation, (ii) their ability to overcome physiological barriers and gain access to affected anatomical regions, (iii) their bioavailability at the disease site and, (iv) their safety profile [13] [14].

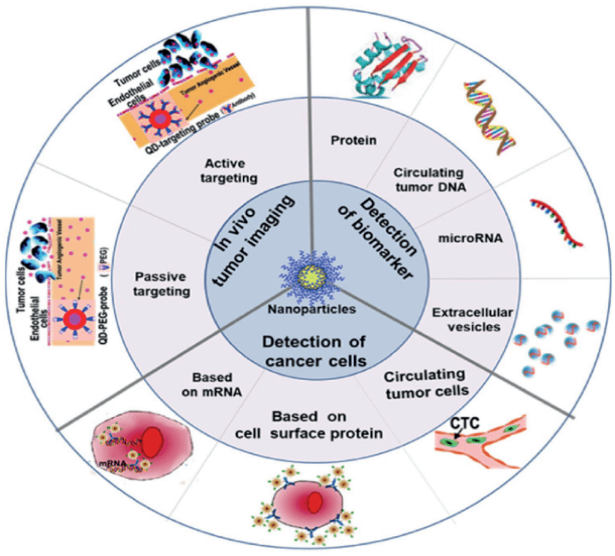

Figure 1: Nanotechnology application in cancer treatment [25]

Nanoparticles

Nanoparticles (NPs) are small systems (1-1,000 nm in size) with distinctive physicochemical properties due to their size and high surface-to-volume ratio. Since NPS have a wide range of applications in optical, biology, and electronic fields, they have been proved to be an efficient

selection for medicine, pharmacy, tissue engineering, environment, energy, electronics, biomolecules, protein diagnosis, cellular engineering, and so forth. These particles have special physical properties such as conductivity, stability, and optical properties, making them ideal for biology and materials science [17]. One of the most important proprieties of nanoparticles is targeting molecules. Therefore, active agents are encapsulated in nanoparticles to reach target molecules in cancer therapy, thus increasing their solubility/biocompatibility, stability in body fluids, and residence time in tumor vasculature. In addition, nanoparticles can be designed to be highly selective for a specific target and respond to a specific stimulus, and release the drug in a controlled manner [15] [16].

The size of the nanoparticle can affect the pharmacokinetics, transport, and cellular uptake of the nanoparticle, which can critically affect the therapeutic efficacy [21]. Another important factor affecting the internalization of nanoparticles into cells is the charge of nanoparticles. Surface modification of nanoparticles can be accomplished by applying various surface chemistries. Cationic particles accumulate more in tumor cells. The surface of a nanoparticle is modified to obtain a positive charge. Besides these modifications, nanoparticle ligand density and nanoparticle flexibility can also be adjusted for better transport and deposition of nanoparticles. These modifications will enable nanoparticles to increase tumor accumulation and localization and prevent target uptake [18].

The NPs can exhibit different bio-physicochemical properties (Figure 2) such as different sizes (1 nm to 100 nm), surface, shape, and soft materials (organic and polymeric) or hard materials (inorganic) can be used. Generally, the NPS are divided into several categories based on their morphology, size, and chemical properties [22].

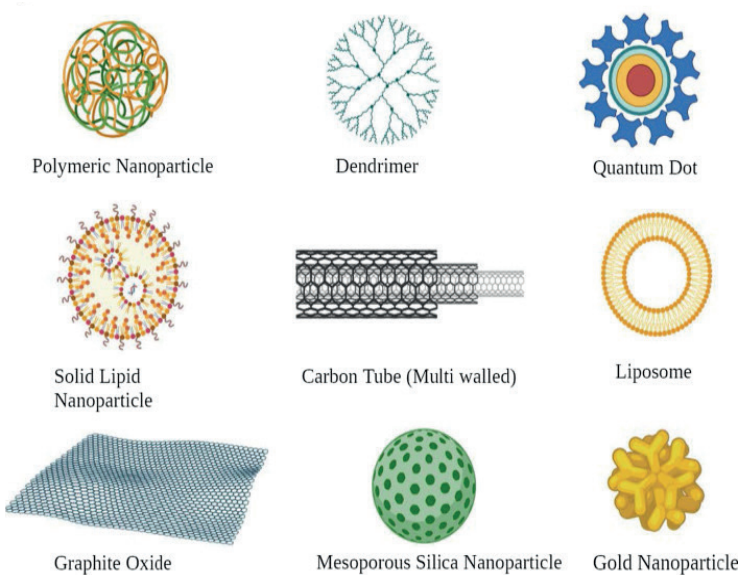

Figure 2: Structure of some nanoparticle drugs [13]

The application of nanoparticles in cancer therapy has been investigated for decades. Among a number of bioactive nanoparticle systems for cancer therapy, polymeric nanoparticles are the most common. PLGA is one of the most widely used FDA-approved polymeric carriers for cancer therapy due to its non-toxicity and biodegradability [19]. In addition to PLGA lipids, it is widely used for the synthesis of PEG (polyethylene glycol). Various bioactive nanoparticles such as liposomes, micelles, dendrimers, gold nanoparticles, iron oxide nanoparticles, carbon nanoparticles, and quantum dots also make them the primary choice for immunotherapy [21]. In addition, inorganic nanoparticles (liposomes, micelles, dendrimers, gold nanoparticles, iron oxide nanoparticles, carbon nanoparticles, and quantum dots) are also widely used as carriers due to their specific targeting, non-toxicity, and biodegradability. All of these nanoparticles can be used to monitor cancer and treat tumors with minimal knockout [20].

Effect of NPs on Cancer Cells

As with any cancer therapy strategy, the main goal is to achieve the desired concentration of therapeutic agent at tumor sites, thereby minimizing damage to normal cells while destroying cancerous cells. With this vision, it requires creating single agents that have the potential to make a significant contribution to the prevention, detection, and treatment of cancer [3].

Cancer nanotechnology is emerging as a new interdisciplinary research area spanning biology,

chemistry, engineering, and medicine and is expected to lead to major advances in cancer detection, diagnosis, and treatment [8] [23]. One of the potential key advantages of nanotechnology for cancer therapy is tumor targeting. The ability to distinguish malignant cells from non-malignant ones and to selectively destroy malignant cells is central to nanotechnology's mission as it relates to cancer therapy [5]. NP-based drug targeting agents have some key features: 1) the ability to remain stable in the vascular system (blood) until reaching the TMJ, 2) evade reticuloendothelial system (RES) clearance, 3) mononuclear phagocyte system (MPS), 4) tumor vasculature It accumulates in the TMJ via the TMJ, 5) high-pressure penetration into the tumor fluid, and 6) reaches the target and interacts only with tumor cells [34]. These properties induce an increase in intracellular concentration of drugs and reduce dose-limiting toxicities [8].

In the cancer treatment process, we have a distinction between malignant and non-malignant cells: passive and active targeting. Passive targeting takes advantage of the enhanced permeability and retention (EPR) effect to increase the concentration of nanoparticles (NPs) in the tumor. Active targeting may involve selective molecular recognition of antigens, often proteins, expressed on the surfaces of cancer cells to localize NPs to malignant cells, or alternatively, exploit malignancy-associated biochemical features such as matrix metalloproteinase secretion. Passive and active targeting can be deployed independently or the two approaches combined. Both strategies take advantage of surface modifications of NPs that minimize uptake by the macrophage phagocytic system (MPS), thereby maximizing the time in circulation [5] [24]

Passive Targeting

Passive targeting represents the preferential extravasation capacity of 10–150 nm nanoparticles from the bloodstream to tumor tissue. Because tight junctions between endothelial cells in new blood vessels are not formed properly in tumors, nanoparticles may preferentially accumulate in tumor tissue. This form of passive nanoparticle entry into the tumor microenvironment is termed the enhanced permeability and retention (EPR) effect [25] [26]. The first macromolecule reported to accumulate in the tumor was identified by Matsuura and Maeda, which was poly (styrene-co-maleic acid)-neocarzinostatin (SMANCS). In later studies, this preferential distribution was attributed to the formation of windows in damaged tumor blood vessels and

poor lymphatic drainage of their association [26].

TME over the EPR effect is a vital feature in passive targeting. It is the main source of energy for cell division and makes the surrounding environment acidic. The reduced pH of the TME can be exploited to use pH-sensitive NPs that release drugs at low pH to the tumor. This type of tumor targeting lacks a specific ligand for certain tumor cell types. EPR effect relies heavily on underlying tumor biology, such as **1)** the extent of angiogenesis and lymph angiogenesis, **2)** the extent of perivascular tumor invasion and **3)** intratumor pressure. These factors, together with the physicochemical properties of NPs, determine the effectiveness of the NP drug delivery system [25] [34].

Examples of passive targeting: Taxanes are one of the most successful drug groups that are used in cancer treatment. Paclitaxel has shown great potency against a broad range of cancers. Breast cancer, lung cancer (small cell and non-small cell), and ovarian cancer are the most treated histologies with taxanes. In 2005, US-FDA approved Abraxane® (albumin-bound paclitaxel, Abraxis Bio-Sciences), which is used for advanced or metastatic breast cancer (MBC) [25]

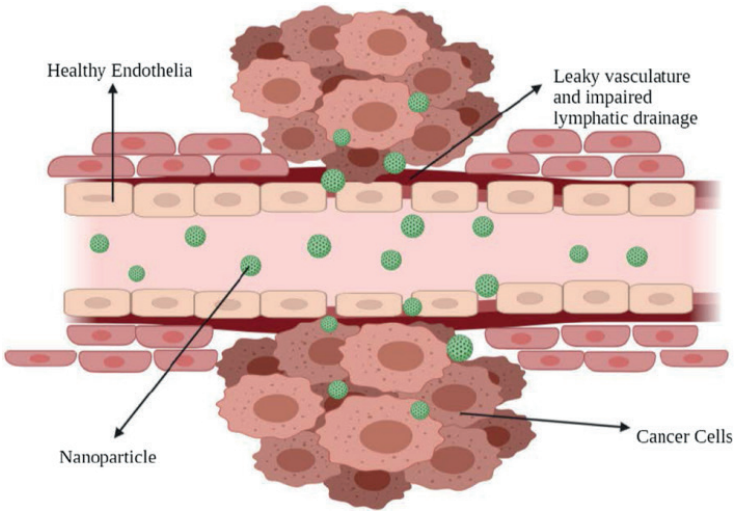

Figure 3: Passive cellular targeting [34].

Active Targeting

This target type is based on the interaction between tumor ligands conjugated on the surface of nanoparticles and cell surface receptors or antigens on cancer cell surfaces (Figure 4). Active targeting is dependent on molecules that are specifically expressed or overexpressed in target

cells (diseased organs, tissues, cells, or subcellular spaces), or specific ligands or molecules, such as transferrin and folate, that bind to receptors. This type of targeting is called ligand-mediated targeting. Here, NPS with ligands with special functions such as holding and uptake need to be near the target for greater affinity. This strategy increases drug penetration by increasing changes of NPs that bind to the cancer cell. Exemplary ligands include proteins, peptides, antibodies, nucleic acids, sugars, and small molecules such as vitamins, etc. may contain [25]. The most commonly used receptors in studies are the transferrin receptor, folate receptor, glycoproteins, and epidermal growth factor receptor (EGFR) [34].

Example of active targeting: EGFR, a member of the ErbB tyrosine kinase (TK) family of receptors, is overexpressed in several types of cancer, particularly by squamous cell histology. Gold NPs with anti-EGFR-PEG-AuNPs and anti-IgG-PEG-Au nanoparticles can be used to target human SCC.

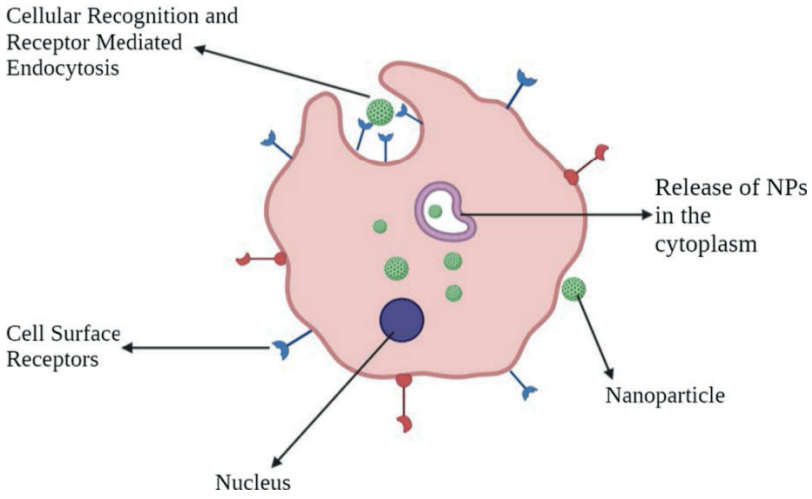

Figure 4:. Active cellular targeting [34].

Mechanisms of NPs Targeting in Overcoming Drug Resistance

Drug resistance is one of the major problems in cancer treatment and management. Drug resistance is a phenomenon that occurs when diseases become tolerant to pharmaceutical treatments. Nanoparticles can also be used to overcome cancer-related drug resistance due to their extraordinary ability to co-encapsulate multiple therapeutic agents [28].

Targeting Efflux Transporters

Efflux transporters belong to the family of "ATP binding cassette (ABC) transporters". The primary function of these transporters is to pump drugs out of the cell and reduce concentration. "P-glycoprotein (P-gp)" is an efflux transporter that is overexpressed by drug-resistant cancer cells. NPS can be used to overcome flow pumps. NPs can bypass efflux pumps, as NPs internalize the cell by 'endocytosis' rather than diffusion and release the drug in the 'perinuclear region' away from active efflux pumps. NPs can also change the control of drug releases, such as using low pH levels and redox as triggers; NPs can effectively bypass efflux pumps [29]. A recent study using COX-2 inhibitors and doxorubicin concomitant NPs has shown to positively affect MDR reversal in breast cancer cells [34].

Targeting an Apoptotic Pathway

The pathways of cancer cells proliferate due to faulty apoptotic mechanisms and increase their survival in addition to drug resistance. The faulty apoptotic pathway gets activated by “deregulation of Bcl-2” and “nuclear factor kappa B (NF-κB). Using a classic process of co-delivery of “Bcl-2 siRNA and chemotherapeutics” by NPs is a way to overcome MDR [30] For example, the combination of ceramide and paclitaxel is a good example. Ceramide restores expression of the major tumor suppressor p53 protein by regulating alternative pre-mRNA splicing. Delivery of ceramides via NPs is an excellent way to correct p53 missense mutation. Because of its potency, a combination of ceramide and paclitaxel has shown significant therapeutic efficacy in cancer drug resistance models. Transfection of the p53 gene by cationic SLNs has been reported in lung cancer cases [31]. A similar study was performed on multidrug-resistant prostate cancer cells using resveratrol and docetaxel encapsulated folic acid conjugated planetary ball milled NPs. In this research, this combination was downregulating anti-apoptotic gene expression while inhibiting ABC transporter markers [32].

Targeting Hypoxia

Hypoxia is an additional condition that favors MDR. Due to the abnormal blood vessels surrounding the tumor and the rapidly growing tumor's increased oxygen demand, some tumor cells are repeatedly in a hypoxic state. The main protein “hypoxia-inducible factor 1α (HIF-1α)” plays an important role. Therefore, targeting HIF-1α or silencing the HIF-1α gene is one

way to overcome drug resistance. NPs containing HIF-1α siRNA can be used to reduce hypoxia-mediated drug resistance. Instead of directly targeting HIF-1α, indirect inhibition of HIF-1α signaling can be used [34].

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Salinity Tolerance in Cotton: Resent Advances in Morpho-Physiological, Biochemical and Molecular Mechanisms

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Abstract

Salinity is a major threat for sustainable agriculture as it can hamper the germination and growth by impairing morpho-physiological, biochemical and molecular processes. The primary effect of salt stress is osmotic stress and secondary effect is oxidative stress which leads to the death of plant. Cotton is primary agriculture product with diverse phenotypic and genetic background having strong impact on economy. It belongs to genus *Gossypium* and out of 53 known species of *Gossypium*, 46 are diploids and the other seven are allotetraploids. In order to respond against salt stress cotton plants have developed different regulatory mechanisms as Na+ compartmentalization into vacuoles in ionic homeostasis, osmotic adjustment, signaling pathways and anti-oxidant defense system. Cotton is moderately salt tolerant crop with salinity threshold 7.7dS/m and can tolerate up to 20 dS/m with an approximately 60% yield reduction under field conditions. Thus, there is still room for the improvement of salt tolerance in cotton. Based on recent papers, the deleterious effects of salt stress on cotton plants are discussed. Furthermore, various salt responsive genes and regulatory factors related to ionic homeostasis and anti-oxidant defense mechanism in cotton are summarized. There is still gap between transcriptomic and proteomic data of identified genes and tolerance mechanism. The reason behind this difference is due to post-transcriptional and post-translation modifications. Therefore, it is essential to analyze the change of proteins under stress conditions with the use of advance technology in order to understand the adaptive mechanism of salinity tolerance in cotton.

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Discipline: Plant Breeding and Genetics

Background

Cotton is a primary agriculture crop that belongs to the genus *Gossypium (Malvaceae)* with broadens phenotypic diversity that contains more than 50 species. Cotton is a diploid (2n=2x=26) and allotetraploid (2n=4x=52) that originated from different genomes [1-3]. According to the investigations, ~1-1.5 million years back from transoceanic hybridization of African species *Gossypium herbaceum* or *Gossypium arboreum* (A-genome) with another specie *Gossypium raimondii* (D5), two cultivated tetraploids species of cotton *G. hirsutum* L. (AD1) and *G. barbadense* L. (egyptian cotton-AD2) have evolved [4]. In cotton, mostly considered that pollen came from the D-genome species (act as pollen giving parent) and A-genome serve as a maternal parent [5, 6]. After molecular verification the cotton tetraploids namely *Gossypium ekmanianum* (AD6) and *Gossypium stephensii* (AD7) were selected taxonomically as new species of cotton [3, 7, 8]. The genetic and phenotypic divergence generated between the domesticated populations through spread and adapted to different ecological land and environmental conditions. That led to form the cultivated hybridized allotetraploid cotton [4]. Globally, four cultivated species of cotton are *Gossypium herbaceum* L., *G. arboreum* L., *G. hirsutum* L. (AD1) and *G. barbadense* L. (AD2).

The major cotton produced by two allotetraploid species *Gossypium hirsutum* with high-yielding characteristics (90%) and early cultivation system [9], extra-long staple fiber source *Gossypium barbadense* (8%) [10] and two diploid species *Gossypium arborium, Gossypium herbaceum* (2%) all over the world [9-12]. *Gossypium hirsutum* and Gossypium *barbadense* play a very important role in cotton production due to their high yield potential and competitive benefit to cotton textiles producers. The cultivated *G. hirsutum* has shown wider adaptability to different agro-environmental conditions with modest fiber quality of cotton. This contributed in approx. 90% of world's cotton production each year. While *G. barbadense* yields remarkable quality of fibers [4]. The origin of *Gossypium arborium* is Indus valley, whereas *Gossypium*

herbaceum (A1 genome, Diploid) originated from South Africa. They both have resistance against hoppers, white flies, and cotton leaf curl virus [12, 13] but *G. arborium* also has thrips drought tolerance and resistance to black root rot [14-16]. *Gossypium klotzschianum* (D-genome diploid, origin-Hawaiian Island), *G. aridum* (D-genome) and *G. davidsonii* (D-genome) have characteristic of tolerance to salinity [8]. Overall, it may be said that D sub-genome of cotton species contains genomic regions which involved in abiotic stress tolerance as well as better fiber quality and higher yield [17].

World Scenario/Pakistan

Cotton is a crop of natural finest fiber which covered biggest textile manufacturing industries devising a strong yearly influence on country economic value of $600 billion all over the world [18]. In recent advancement in the perennial growth habit of wild cotton, converted it into an annual crop. Primarily, cotton is planted for fiber use in textile which is the main function of seed epidermal cells [19]. However, cotton seeds are also essential for edible oil production which takes part to a large proportion of the oil needs and fulfill the major part of the edible oil prerequisites. In Pakistan, domestic oil production is nearly 78% from cotton seed [18]. The seed residual cover is enriched with many organic compounds that can be used in livestock feed purposes [20, 21]. Globally, cotton is grown in many countries and it helps to improve country Gross domestic product (GDP) through the endowment of fiber, seed edible oil and number of other things [18]. Generally, cotton production is about an annual total of 25 million tons and its value is more about 12 $billion worldwide [19].

According to the 2019 report survey, India, China, USA, Pakistan, Brazil, Australia, Uzbekistan and Turkey are included in the list of top cotton producing countries [22]. India is at first number in total world cotton production in spite of low yield per acre. Out of 35 provinces, China planting cotton in 24 provinces with 99.5% area used in cotton cultivation. Hence this shows that cotton is the primary crop of China. In cotton export, the USA is most prominent country by producing major proportion of cotton. Pakistan is also known for major production and consumption of cotton among all other countries [23, 24]. About 14% increased in global cotton production occurred during the year 2018 on account of increasing major cotton-producing countries. The United States of America (USA), China and Turkey estimated to increase 20% from the production of last years. This comparatively increased in the production is due to

increase in area for cotton plantation [25]. According to an investigation, during the year 2017–2018, global purchase of cotton were US $49.9 billion [18]. On area aspect, maximum cotton (65.5%) purchased by Asian countries and left apportion imported by Europe. China ranked first in cotton import. Similarly, Pakistan is at the 6th position in cotton import market with import value of 621 thousand bales [19]. Although Pakistan is placed among globally top five cotton producing countries, on the other hand its yield lag behind to other top most countries due to low yield per unit area and increasing the cotton import. The reasons behind this lack are different and numerous.

Cotton is mostly cultivated in warm areas of Sindh and Punjab. The cotton cultivation in Punjab is approximately 80% whereas the remaining cotton is grown in Sindh. The position of cotton is shown from the fact that it provides occupation to the larger community of people in Pakistan from 1.3 million farmers in fields to establishing the fiber textile industries (power looms and oil seed factories) as well as large number of knitwear and garment units on domestic and non-domestic level. So, the commodities of cotton has about 10% share in country GDP and above 50% in the foreign reserves market [22].

Factors Responsible for Low Yield in Cotton

With frequent extreme weather conditions and climate change, particularly abiotic stresses that are mainly associated to the cotton yields. Climate change reduces production of any crop because of stress factors such as heat, drought and salinity etc. It is difficult to deal with these factors as they develop a continuous risk to cotton production. Abiotic stresses like drought and salinity can be achieved genetically or agronomically [18]. Other important reasons beside climate change that cause poor yield in cotton viz. irrigation with salt containing water that changes soil nature, insufficient fertilizer use, ineffective control of pests and drastically affected virus diseases, late sowing of cotton, cold and heat stresses [22].

There are different factors that affect cotton yield. Among these, climate changes are main factors responsible for causing low cotton production as previously explained, but soil salinity is the prevalent problem that is also caused by climate change. Salinity, sodicity, alkalinity and waterlogging have negative effects on the productivity of the cotton cultivated area. Low yield, poor plant growth, and germination are main constraints that are affected by salinity, alkalinity

of soil that limit cotton growth at the early stages of development [26, 27]. Salt stress impose drought in cotton that act as a osmoticum [28]. Cotton seed germination is an important phase but unfortunately it is also very sensitive stage for harsh climate conditions. These environmental factors can hinder seed germination by reducing plant water uptake ability and impose drought and deploy it from nutrients by disturbing the ions uptake mechanism [29].

Cotton is native to hot climates, but increase in temperature affect negatively to early boll development. The optimal thermal temperature for Upland cotton is 23-32°C effective for metabolic activity [30]. In the same way Cotton is sensitive to low temperatures influence its germination and growth rate. Usually cotton plant needs abundant sunshine for its proper growth [31].

Rapid climate changes are dangerous to the maintenance of cotton production system in Pakistan [22]. To overcome these unexpected climate changes that effect on yield, important task is breeding of abiotic tolerance cultivar in order to maintain productivity. Similarly, over the period of time cotton production area has declined due to moving of cotton cultivated area to other crops such as sugarcane and maize by special policy offered by government of Pakistan, has ultimately discouraged cotton growing farmers [22].

Pakistan lags behind the target area and production because of these environmental as well as biological factors. According to the Dawn news the Punjab cotton production in report of the 15 Sept (2019) recorded as fall from the yield comparative to last year 2018 by 0.598m bales against 0.980m bales. This tragic condition has direct clash with the Pakistan GDP by exerting pressure on its foreign exchange rate because huge quantity of about 3-4m cotton bales will have to be imported in order to overcome the loss.

Stress

Factors that interfere with the genotypic expression potential are known as stresses. There are two kinds of stresses. Biotic stress includes pathogens mainly virus diseases of cotton CLCV, weeds, pests and abiotic stress particularly water deficit effect, salt stress, high temperature are an important production limiting factors in some major cotton growing countries [30]. About 50% of plant productivity is affected by these abiotic stresses [32]. out of all these types of abiotic stresses salinity is our main concern.

The excessive concentration of salt (above from threshold i.e. 3-4 dS/m) in the soil, water and then plant is called salinity [33]. Exposure to salt stress triggers many common adverse physiological & biochemical changes in plants leading to yield reduction. As a perceptive to abiotic stress tolerance, cotton is proposed as a medium salt tolerant crop with salinity threshold 7.7 dS/m [34] it might be exploited for soil recovery and produce better-quality land for other crops production [35]. But in sensitivity to salt and drought stress its genotypes differ significantly [36]. Thus, it is very important to screening of salinity tolerant genotypes to maintain area of productivity on subsidiary land under saline conditions. It would help to meet the requirement of fiber, feed and industrial raw material for growing population and biological reinforcement of salt-affected soils also for other crops.

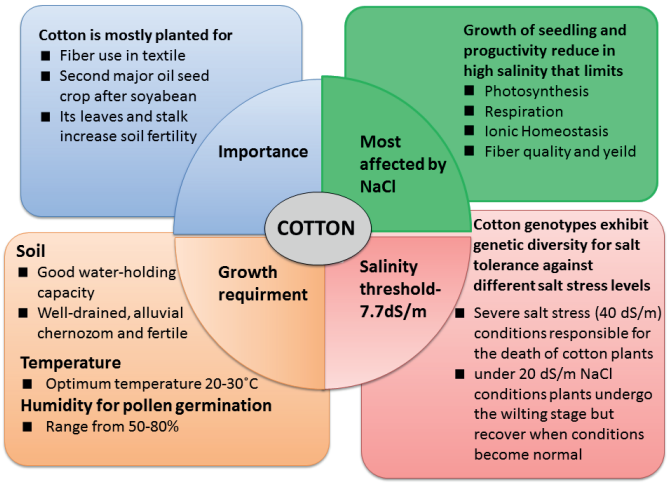

Figure 1: An overview of cotton plant requirements and salinity effects

Soil salinization is rapidly increasing day by day that decrease average yields of most major crops like wheat, rice and cotton over and above 50 percent on a global scale [37]. Salt in soil caused damage by salinity or its related condition of sodicity (high proportion of cations especially sodium concentration in soil) over 800 m ha of area throughout the world. Currently out of 230m ha of irrigated land, in which 45 m ha area is under the influence of salt [38].

Salt stress activate cellular desiccation, that result osmotic stress along with the release of water from cytoplasm into the outer cell spaces cause water decline in cytoplasm as well as vacuolar volume [37]. Another drastic damage due to salt stress is that it generates reactive oxygen species (ROS) which cause the big damage to the cellular structure and metabolism by

producing free radicals [39]. Generally cotton growth and plant development comprising plant height, fresh and dry weights, plant weight, root to shoot ratio, leaf area and canopy development, and other physiological parameters like photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (St), overall yield and primarily fiber quality severely hindered by salinity [30]. The cotton fiber consumption is increasing as human population grows. By means of conventional breeding and molecular breeding method by utilizing marker-assisted selection (MAS) researchers working on developing new cotton varieties of high yield and tolerance under salt conditions [40].

Salinity

The reasons behind high salt concentrations in soil are complicated and multifactor such as arid climate structure, high under-ground water level, seawater infiltration and many others [41]. Over the period of time, the residues from the disintegrating of rocks and minerals gathered in the soil under arid and semi-arid climates, resulting of salt containing soils (such as saline or sodic soil) [33]. Salt affected soil can be divided into saline (NaCl), alkaline (NaHCO3 and Na2CO3) and salt-alkaline soils (containing both alkaline and salt molecules) [42]. Salt-alkaline nature of soil is due to excessive salt ions and having high pH by alkaline stress [43]. There are many factors responsible to increase salinity issues viz low precipitation, high rate of evaporation from surface area of land, disintegration of intrinsic rocks, saline water irrigation and ineffective agronomic approaches [33]. About 20% of the global land is under the influence of salinity [44].

Plants on the basis of advancement in their evolving mechanisms divided into two types known as halophytes (resist in salt stress) and the glycophytes (that can't endure salinity and ultimately die) [39]. Halophytes have developed distinctive mechanisms that help them to withstand in Salinity, for instance salt glands (GTs), salt bladders, and succulents, in order to excrete Na+ of their organizational structures [45-47]. The instant exposure to salinity from seconds to minutes effect on root growth reduction because roots are the first place to identify salinity and leaf elongation rate then partial recovery occur immediately because plant maintain their homeostasis but if salt stress prolong for hours, so the persistent reduced rate of leaf and root elongation was noted. If exposure to salt stress continue from days to weeks reduced the rate of leaf emergence, reduced branches and tillers formation reduce plant height and also wilting was

observed. Salt stress has more negative impact on leaf growth as compared to root growth [48].

Plants acquired important protective responses which assist them to survive and reproduce in unfavorable situations [41]. Calcium (Ca^{2+}) discernment, reactive oxygen species production, and protein phosphorylation are some signaling events during initial phases of plant responses. For better performance of plant under salt stress, naturally evolved adaptation responses in physiological, molecular, and cellular levels are most important. For an ideal plant growth and development these important adaptations such as; osmotic adjustment so as to minimizing the osmotic stress, reduced stomatal conductance, removal of Na^+ from older leaves, maintenance of K^+/Na^+ ratio (K^+ concentration in cell cytoplasm), transpiration rate and increased defense systems against reactive oxygen species are significant under salinity [33].

Plant may suffer from physiological drought due to increase amount of soluble salt in soil which decline water intake in plant as result of root zone deposition with large amount of salt [49]. In this way osmotic potential of plant may reduce, ion toxicity cause disruption of cell metabolic function. Many physiological changes like membrane disruption as a consequence of osmotic stress, decrease in photosynthesis rate (Pn), and biochemical changes also occur in the plant i.e. reactive oxygen species (ROS) production, and scavenging of antioxidants [50]. The large amount of sodium ion (Na+) has affect on potassium (K+) ion concentration as an antagonistically [51]. While on contrary, salt stress has adverse effect on P, K^+, Fe, Ca^{2+}, and Mn. So, the salt tolerance mostly referred as the plant ability to achieve osmotic balance by regulating cellular Na^+ ion balance inside the cell so as to lessen the cytotoxic effects of the ions [32].

Cotton

Germination, emergence [52] and seedling growth [53] are particularly salt-sensitive stages (

). The main factor under salt stress condition in cotton is the positive ion (Na^+) instead of (Cl^-) [54]. Under salinity stress the Na^+ content in the shoots (cotyledon, leaves, and stems) greater than in the roots. In leaves different types of cells having different capacity to sodium accumulation that is more in epidermal cell rather than mesophyll cell and this was mainly noted in sensitive genotypes [55]. Similarly, the salt-tolerant genotypes have aptitude of maintaining K^+/Na^+ ratios. For osmotic adjustment, the Na^+ sequestration into the vacuole is

very important in order to minimize the Na^+ concentration in cell cytoplasm [56]. For this purpose, Na^+/H^+ antiporters [57, 58] V-ATPase and V-PPase (vacuolar pyrophosphatase) these two H^+ pumps are involved in Na^+ compartmentalization into vacuoles [59]. Similarly the salt-tolerant genotype has more capability of more Na^+ repossession into the vacuoles.

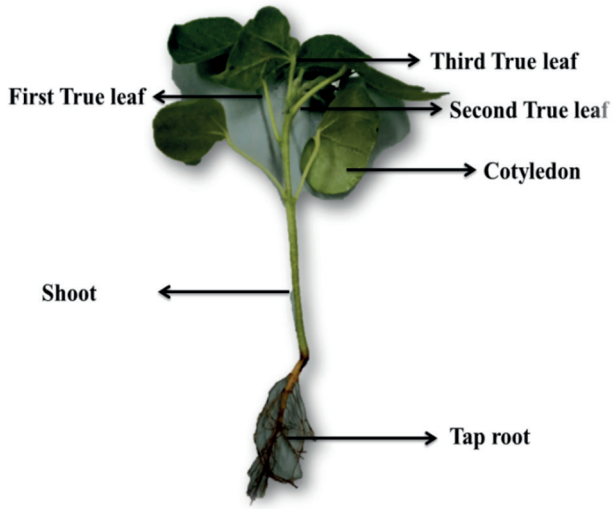

Figure 2: Cotton plant seedling at 4th true leaves stage

High concentration of NaCl mainly causes ion toxicity, osmotic stress and mineral disruption (such as those of K+ and Ca2+) in plants [32, 60]. High salt level has severe influence on main root growth of cotton seedlings but supplemental Ca^{2+} minimize Na^+ inflow and improved root growth [53, 61]. Thus the supplemental Ca^{2+} has a protective effect on root growth in high salt stress and also related with maintenance of K^+/Na^+ ratio with improved Ca^{2+} status [61]. Another process of Na^+ compartmentalization in upland cotton is a salt secretion. GTs (indicated as glands) are used for salt secretion resolves which are present on the surface of cotton leaves secrete different substances, e.g., hydrated carbonates, cations and lipids etc. [62] which involve in plant defense and have protective functions [63]. Some halophytes also have developed salt glands also referred as salt hairs on their surface of leaves for the aim of salt secretion when exposed to salt stress to minimize salt accumulation. Salt tolerance in halophytes is highly correlated with the capacity for secreting salts (by salt glands) [64].

Cotton crop yield can be increased on salt-affected soils by improving salt and drought-

tolerance of existing varieties or introducing new high yielding salt and drought-tolerant varieties using modern ways of breeding such as biotechnology or molecular engineering. To accelerate the efforts in attaining this goal, screening of existing cotton germplasm is required for salt and drought-tolerance. Beside these, several management strategies of recent time have comprised on a scientific base to improve plant growth proficiency in salt stress condition.

These approaches are genetic modification (by inserting genes, mutation, etc.) identification of such valuable traits in genome, sequencing of that traits related gene, microarray analysis would be done, and then transformation into the plant along with agronomic strategies to decrease salt stress by soil recovery through water and nutrient management, some other strategies like seed priming with $CaSO_4.2H_2O$ and use of hormone regulators to generate homeostasis in hormonal synthesis during salt stress [33].

Morphological and Physiological Responses

Excessive load of salts in soil has an adverse effect on morphology and physiology of plants as well as dramatically falls in crop quality and its production rate [65, 66]. This detrimental effect of salt (NaCl) on cotton differ in accordance with the change of salt concentration (i.e. 10dS/m, 20dS/m etc.), depending upon the time period of salt exposure (that how long it will be) and growth stage of plant in which exposed to stress (i.e. germination or emergence). As a result of these factors, less and delayed cotton seed germination, stunt vegetative growth of plant and finally reduced its yield. The main effects of high concentration of salt at cellular level are hyperosmotic stress (that reduces the stomatal conductance) along with ionic stress, absence of essential nutrients and secondary effect involve oxidative injury to proteins (enzymes) and membrane stability [67, 68].

During seedling and vegetative stage cotton plant undergoes to reduction in many physiological parameters [69-72]. There is also considered as the growth rate directly related with stomatal conductance, higher the stomatal conductance higher will be the CO_2 absorption and energy production. In salt stress condition plant undergoes to cellular injury occur in transpiring leaves because of large amount of salt accumulation. Perilously cotton's crop production is extremely reduced because of poor seed germination under salt stress conditions [73]. In contrast, plants which have been exposed to salt during their early (seedling) stage produced less as compared

to those maturing cotton plants which have stressed in their vegetative and adult plant stages (i.e. flowering and boll formation) [73].

The longer exposure to salt is more profound with negative effects on cotton. In view of this, mature cotton plants may exhibit reduction in fruit node number, late fruit initiation along with fiber length and strength which affect the quality and quantity of cotton yield. This happened particularly when cotton is under prolonged period of salt stress conditions [26, 74-76]. By comparative study, certain varieties have been regarded as salt tolerant for instance Acala 1517-88 and Acala 1517-SR2 [77], NIAB-78 [78], and MNH-93 [79]. Salt in soil hindered the absorption of other necessary ions therefore reduction in ions concentrations within roots and leaves was observed [80-82]. However cotton is referred as salt tolerant crop after barley with salt stress limit 7.7dS/m and its yield falls by 5% per unit dS/m with the increase of stress limit [83]. Thus, the 200mM NaCl (20 dS/m) stress treatment would induce an approximately 60% yield reduction underneath the field conditions [84].

Netondo et al. [85] proposed that a shorten in leaf area would disturb the photosynthesis that leads toward reduction in biomass deposit. Salt stress inhibits the uptake of K^+ due to competition between Na^+ and K^+. Potassium is associated with turgor regulation process but its reduction in cotton may also contribute in decrease height of plant and leave expansion in salt stress plants. Abd-Ella and Shalaby [86] described that excessive load of NaCl had not been changed the amount of Ca in cotton leaves. Though Ca concentration has found to be increase in some salt-tolerant cotton genotypes [26]. Elements like Zn and Mn concentrations tend to be increase whereas Fe and Cu decrease after salt stress.

Halophytes are adapt to salt stress as well known but they evolve some mechanisms that help their survival by preventing or avoiding entry of salt (having excretion mechanism), sequestration of salt ions like Na^+ by moved into the vacuole, salt glands (Glandular trichomes-GT or salt hairs) and de-toxification of reactive oxygen species via osmolytes and anti oxidant enzymes like SOD and POD [67]. Theoretically, sensitivity against salinity might be due to large amount of salt levels present inside the germination zone of soils. As a result of low humidity, high temperature and wind pressure cause evapo-transpiration and capillary rise of ground water which create the accumulation of soluble salts by reducing water level [67]. This course of action makes plant more sensitive to salt stress and cause morpho-physiological

changes that ultimately affect the cotton production. Hence, screening of cotton genotypes against salinity at their initial stages of development in cotton is beneficial to breeding programs to sustain country's GDP and economy.

Biochemical Responses

Salinity stress is multigenes reaction, a numerous procedures are related to tolerance mechanisms are under influenced of salinity; like reactive oxygen species and anti-oxidant defense mechanisms [87]. Salt stress also affect the metabolic activities of plant tissues by over production of reactive oxygen species e.g. superoxide O^{2-}, hydrogen peroxide H_2O_2, $^{\bullet}OH$, and $^1O_2$ that damage cellular structures and enter the plant into oxidative stress [32, 88]. Increase of reactive oxygen species (such as O^{2-}, $^1O_2$ and $^{\bullet}OH$) in the cell oxidize different biochemical compounds like: proteins, lipids, DNA and RNA. Also, extreme UV light has been shown to induce alterations in efficacy of electron transport chain and as a consequence of higher ROS production in plant cells, cause the generation of ROS scavenging enzymes in plant defensive mechanism [89].

Plants possess a various extensive anti-oxidative mechanisms in order to regulate the reactive oxygen specie for instance ascorbate peroxidase (APX) pathway and superoxide dismutase (SOD) pathway for protection against reactive oxygen destruction. SOD pathway is a first course of action against ROS. It employed as ROS scavenger to cope with salt stress by converting O^{2-} into H_2O_2 and O_2 in roots [90]. Similarly H_2O_2 could get H_2O and O_2 by reduction reaction in catalase (CAT) pathway confined in peroxisomes organelle [88]. POD is a heam-containing glycoprotein which catalyzes the H_2O_2 in reduction reaction by using different electron donors for example phenolic compounds and secondary metabolites [91, 92]. Glycine betaine and proline work as a scavengers of ROS and osmolytes during the salt stress situation [93].

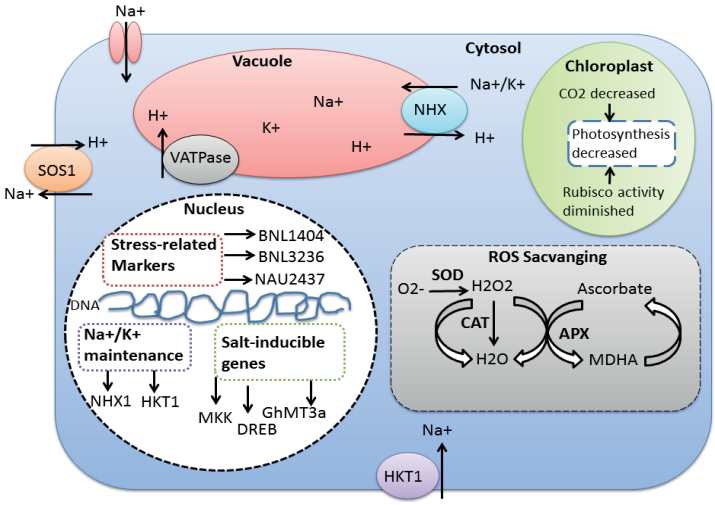

Figure 3: Schematic representation of salt responsive mechanisms at cellular level in cotton

The superoxide dismutase (SOD) activity is enhanced under salt stress. Likewise, melondialdehyde (MDA) content is increased with rising of lipoxygenase (LOX) activity as LOX act as a catalyst in the production of fatty acid. Anti-oxidant enzymes activity like peroxidase (POD) and catalase (CAT) lower in salt sensitive cultivars than the tolerant cotton genotypes. In addition, cotton genotypes which are tolerant to salinity exhibit greater activities of SOD, ascorbate, POD and glutathione reductase with less catalase (CAT) activity as the amount of salt increases [18].

In cotton the expression of SOD enhanced with respect to the rise in NaCl concentration. Whereas POD activity higher up to 53% in tolerant cultivars. Higher POD activity has improved photosynthesis which shown the part of anti-oxidants defense system to alleviate salinity stress [94, 95]. Ascorbate/ascorbic acid is another essential non-enzymatic anti-oxidant which one's amount more in cytoplasm and cell chloroplast when it exposed to salt stress. Ascorbic acid has ability to keep maintain the photosynthetic apparatus in chloroplast under salinity. In many genotypes more ascorbic acid in their cell is seems at early growth stages as indication of tolerance [96].

Molecular Responses

For abiotic stress tolerance in cotton plants a numerous molecular methods are found and with

the recent advancement along with biotechnology techniques many approaches are developed. So the molecular mechanisms comprise of stress tolerance or stress avoidance events. Plant tolerance is complex trait or multi-genic response which is influenced by both genotypic (genetic/gene) and phenotypic (environmental) effects. Genes that are involved in these molecular phenomenon or related to specific trait can be labeled with the advantage of molecular mapping methods [97, 98]. Molecular mapping is important in mapping of stress-associated QTL/genes and has been utilizing in cultivating new resistant genotypes via marker-assisted selection (MAS). These methods are reflected very applicable in generating stress tolerant cotton cultivars [99]. As mentioned above, resistance in plants is multigenic response certain markers are associated with more than one traits signifying that one gene may control multiple phenotypes [100].

Peleman and van der Voort presented first molecular breeding design [101] of three steps. By following this method in the first step; localized the agronomically relevant marker in genome by mapping, in second step; assessment of allelic variation of that gene and third step is; breeding by design. In conventional breeding there are some limitations like lack of genetic variations, linkage drag and segregation distortion [102-104]. But this breeding design increases the predictability in conventional breeding which is on the basis of phenotypic selection by experience leading toward molecular breeding by prediction nevertheless with efficiency and effectiveness [105].

With more advancement and successful application of molecular markers and MAS in plant breeding, breeders are used to assess genetic control on complex quantitative traits. As an illustration, next-generation RNA-sequencing identified many salt related genes in the roots and leaves of *G.davidsonii* plants. These genes illuminated the function of salt overly sensitive (SOS) and reactive oxygen species (ROS) of signalling pathways in *G.davidsoni* [106]. Similarly another salt tolerant cotton specie *G.klotzschianum* plants were exposed to extreme stress with high salt concentration (300 mM NaCl). Likewise *G.davidsoni* these gene are also functionally annotated in ROS and SOS signaling pathways along with hormone biosynthesis [107]. Genome wide SSR markers for marker trait associations of salinity tolerance in cotton e.g. BNL310 (D6), NAU478 (D8), and BNL3140 (D9) have been confirmed to associated with salt treatment [17] and BNL1404 associated with RPOD (relative POD),

BNL3436 with RSOD and NAU2437 with RMDA [108].

There are many salt responsive genes have been identified that are related to different stress responsive mechanisms for example salt stress signaling pathway (e.g. guanosine 5'-triphosphate-binding proteins [109]), antioxidant defense mechanisms (e.g. Ascorbate peroxidate (APX) [110, 111], glutathione reductase (GR) [112], and glutathione S-transferase (GST) [113]), protein turnover (e.g. heat shock protein (HSP) [114], cytoskeleton (e.g.actin [115]), and amino acid metabolism (e.g. glutamine synthetase (GS) [116]). For ionic homeostasis, ABC (ATP-binding cassette) transporters (gi|224130846) in cotton roots has been reported [117]. Which involved in energy production by ATP utilization mainly for the transportation of micromolecules [118] and secondary metabolites (alkaloids, terpenoids) related to stress [119]. In addition to ionic homeostasis regarding Na+/K+ maintenance other genes i.e. HKT1 that control Na+ influx and K+ uptake by the release of HKT (high affinity K+ transporter) [120] and NHX1 which involved in vacuolar Na+ sequestration [57] are known to play a significant role in salt stress tolerance in cotton.

Table 1: Trait associated markers for the selection of salt tolerant genotypes in cotton

Sr. no.	Marker	Type	Species	Product length	PCR condition	Associated trait	F/R sequence	Ref.
1	HAU1857	SSR	*G. spp*	298bp	AT =57	SDW	F:GTTGGCCGAGAAAACTAAGA R:CGTCGCAATGTGTTCTAATC	[104]
2	CGR6949	SSR	*G.hirsutum*	190bp	AT = 55	SDW	F:TACTGACCAAGCGCCTTCTT R:TTCTGCTACTGTCTCCGTTTGA	
3	CGR5561	SSR	*G.hirsutum*	128bp	AT = 55	SDW	F:CTGAAGGCTGAGCACAAACA R:GCTTATCGTAGCTATTCACTTCTAC	
4	NAU5277	SSR	*G.hirsutum*	150bp	AT = 57	SDW	F: ATCTAACCCCTTTTGCCTTT R:CGACAAATCCAAGATGACAA	
5	NAU3358	SSR	*G.raimonndi*	246bp	AT = 57	SDW	F:ACCAAATCTCTTGGAGGTGA R: CCACGGAACTTTTCTTTTTG	

6	NAU7049	SSR	*G.barbadense*			RSOD	F: AGGTACCTCTCCTGACTCT R: AATCTTCTTGAAATCGAAC	
7	NAU2437	SSR	*G.hirsutum*	234bp	AT = 57	RMDA	F:CTTGGAAAAAGGAAGAGCAG R:TTAAAAGACCAAAGGCAAGG	
8	BNL3436	SSR	*G.hirsutum*	196bp	AT = 55	*RSOD(RPODand RMDA)	F:AACATAGCCTACCATTGCCG R: TTGTTTGCCAAATTTGAAGC	[108]
9	BNL1404	SSR	*G.hirsutum*	230bp	AT = 55	RPOD	F:CGAGAGCCCACTAACAGAAA R: CCATTTGTTTTTTCCCCCTT	
10	BNL3103	SSR	*G.hirsutum*	190bp	AT = 55	DPW, DRW	F:ACTTTGAGATATTGTTATTCTACCCG R:TCGAACAATTACGAATCAAATG	[17]
11	BNL3140	SSR	*G.hirsutum*	107bp	AT = 55	RSR,DRW	F:CACCATTGTGGCAACTGAGT R:GGAAAAGGGAAAGCCATTGT	

Moreover, the up regulations of many other genes under salt stress like ABCB21 (Gh_A12G1090), ABCG36 (Gh_A10G0583, Gh-A05G1089) and ABC2 (Gh_A09G1286) are reported in Gossypium hirsutum [121] as well as salt stress-inducible genes e.g. Na+/H+ antiporter [122], DREB [123], ERF [124, 125], NAC [126], GhMT3a [127], MPK [128], MKK [129], and ZFP [130] are also identified in cotton. Many salt resistant genes have been discovered in model plants but few salt stress inducible genes identified in cotton as mentioned above. The reason behind this gap is due to difference between transcriptomic and proteomic data that give non-similar results due to post-transcriptional and post-translation modifications. Therefore, it is essential to analyze the change of proteins under stress conditions in order to understand the adaptive mechanism of salt stress tolerance in cotton.

Conclusions

Salt stress causes drastic effects on cotton plants which limit its yield as well as fiber quality. Salinity stunts the plant growth due to unequal supply of essential nutrients, water deficit effect, osmotic and oxidative stress which ultimately reduce the yield. In spite of yield, it also affects

the quality of harvest product. Mostly the short term solutions failed to resolve this critical situation. Hence it is imperative to resolve this problem by producing genetically tolerant varieties in order to overcome the losses. In order to enhance stress tolerance, extensive research at cellular, metabolic, physiologic and molecular level is needed. By taking advantage of latest technology in genomics, transcriptomics, proteomic and metabolomics for the improvement and development of salinity tolerance mechanisms researchers are focusing on it in order to meet future demand of cotton fiber. It will be important efforts with respect to molecular breeding for the development of elite cotton verities with improved salinity tolerance in view of climatic scenario.

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The Neuroprotective Features of Melatonin in Alzheimer's Disease

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Abstract

Alzheimer's disease is a progressive neurodegenerative condition, and its defining feature is amyloid deposits, neurofibrillary tangles, and neuron loss. Many former studies estimated the effects of antioxidants in alleviating the symptoms of Alzheimer's disease (AD). *Melatonin* is a lipophilic hormone primarily released at night by the pineal gland. It has been shown that Melatonin possesses powerful features as a potent antioxidant and free radical scavenger. Melatonin has also been shown to promote other antioxidant mechanisms that may improve cognitive function and inhibit amyloid deposition. Several studies have also shown that Melatonin possesses a favourable impact in inhibiting tau protein hyperphosphorylation. Ought to these findings, many studies suspect a tremendous impact of Melatonin, especially in halting Alzheimer's disease. In this review, we will discuss in detail the main effect and the suspected things in the future.

Keywords: Alzheimre disease,Melatonin,Antioxidants,Cholinergic.

Discipline: pharmacology

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Introduction

Alzheimer's disease (AD), one of the most common neurodegenerative diseases, causes several progressive problems, including memory loss, behavioural changes, functional decline, and learning disabilities [1]. The main cause of dementia is AD, and it is predicted that 40 million individuals worldwide have dementia. From now until about 2050, this figure is expected to double every 20 years [2]. Alois Alzheimer, a German psychiatrist, was able to describe the first instance of a 51-year-old lady who appeared with several increasing memory loss and mental disorders in 1907 [3]. Neurofibrillary tangle was the hallmark of Alzheimer's disease at that time, distinguishing it from other progressive and fetal neurodegenerative diseases, such as senile dementia [4]. Emil Kraepelin referred to his psychiatric handbook's 8 edition for the first time and referred to this medical condition as Alzheimer's [5,6]. In the hippocampus, senile plaques made of amyloid beta (A) and neurofibrillary tangles (NFTs) made of phosphorylated tau protein are thought to be the primary pathogenic cause of Alzheimer's disease (AD) [7]. Three forms of AD exist: familial, sporadic, and late-onset [8]. APP and presenilin gene mutations are the major causes of familial AD, but the aetiology of sporadic AD is complicated and includes genetic, environmental, metabolic, viral, and other variables [9]. Statistics indicate that APOE is associated with between 40 and 50 per cent of EOAD and 80 per cent of LOAD. The generation, hydrolysis, and removal of Aβ are impacted by APOEs [10,11]. Three isoforms of the polymorphic protein apolipoprotein E (APOE) exist APOE2, APOE3, and APOE4, with APOE4 being the highest genetic risk factor for SAD [12,13]. The release of phosphorylated tau is encouraged by endogenous APOE4 expression in stem cell-derived neurons, making neurons more vulnerable to damage and calcium dysregulation. The primary lesions of AD were associated with intracellular Tau neurofibrillary tangles (NFTs) and extracellular amyloid plaques [14]. The primary ingredient of amyloid or senile plaques (SPs) is an extremely insoluble and proteolysis-resistant peptide fibril produced by the cleavage of -amyloid (Aβ). The two enzymes β-secretase and γ-secretase are responsible for breaking down the precursor protein APP (amyloid precursor protein). Nevertheless, If the enzyme α -secretase instead of -β secretase initially acts on and cleaves APP, Aβ is not produced [15]. The "amyloid hypothesis" states that the creation of Aβ in the brain commences a chain of a cascade that eventually results in the clinical presentation of AD. Numerous elements are involved in this cascade, such as hyperphosphorylation of tau and localized inflammation, oxidation, and

ecotoxicity (excess glutamate) [14]. The tau protein, a microtubule-associated protein, interacts with microtubules in cells to help the neuronal transport system. The stabilization of axons by microtubules plays a crucial role in the growth and operation of neurons. Unusually hyperphosphorylated tau folds into intraneuronal tangles, creating insoluble fibrils [15]. It was previously believed that tau hyperphosphorylation was a downstream event of amyloid(Aβ) deposition and that it functions in a parallel pathway with Aβ to cause AD and increase the toxicity of both processes [16]. Although there are treatments that can help with the symptoms of Alzheimer's disease, there is currently no cure for the condition. the available treatment is included acetylcholinesterase inhibitors (AChEIs) are based on the cholinergic hypothesis, which claims that memory, learning, attention, and other higher brain processes diminish due to the progressive loss of limbic and neocortical cholinergic innervation in AD. The AChEIs are therapeutically effective in postponing cognitive deterioration in AD because they improve the availability of acetylcholine at synapses [17]. Memantine, a low-to-moderate, noncompetitive NMDA receptor antagonist, is another medication for treating moderate to severe (AD) [14,18]. Memantine reduces the dangerous consequences of pathologically increased glutamate levels that cause neuronal dysfunction by binding preferentially to open NMDA receptor-operated calcium channels and reducing NMDA-mediated ion flow [19]. Oxidative stress, which comes from an imbalance between the production of free radicals and antioxidant defences, contributes to neurodegeneration [20]. A methoxyin-43 hormone called Melatonin is released by the pineal gland when there is no light. It was formerly thought to be only engaged in hormonal processes, such as circadian rhythm modulation, sleep homeostasis, vaso-activity regulation, and the development of reproductive glands. Melatonin controls the sleep-wake cycle and serves as a free radical scavenger, antioxidant, and immuno-modulating hormone [21]. Melatonin's antioxidant qualities are linked to its neuroprotective effects in many degenerative diseases [22,23].in this review; we will discuss Melatonin's impact on vascular alteration in neurons, the cholinergic system's deficit, and tau and amyloid beta proteins.

Melatonin İs A Powerful Antioxidant

Melatonin is a well-known compound that has been around for three billion years. Its composition was first determined in 1958 [22]. The pineal gland, driven by the internal biological clock, and SCN (the suprachiasmatic nucleus), which controls the circadian rhythm

in all animals, including humans, release the majority of Melatonin. Since Melatonin is a light-sensitive hormone, photoperiod is crucial for both melatonin synthesis and the timing of its secretion. The pineal gland receives a neuronal signal from the SCN, which serves as a vital relay centre, and the pinealocytes are affected by the central and peripheral sympathetic nervous systems [23]. The first step is light absorption by the eye's retina, which creates a signal and travels down the retinohypothalamic tract to the SCN in the hypothalamus, which is then mounted in the optic nerve. Following the paraventricular nuclei, this signal travels via the spinal cord's intermediolateral cell column before reaching the superior cervical ganglion (SCG). The SCG transmits signals to the pineal gland, which uses norepinephrine to create Melatonin from its precursor, serotonin [24,25]. From a biochemical perspective, Tryptophan serves as the precursor for the metabolic process that produces Melatonin. Three phases comprise the synthesizing process: hydroxylation, decarboxylation, and acetylation. In the initial step, Tryptophan is converted by the enzyme tryptophan hydroxylase into 5-hydroxytryptophan (5-HTTP). The second step involves removing the carboxyl group from the side chain before using aromatic amino acid decarboxylase to convert it into serotonin (AAD). Serotonin is acetylated to N-acetylserotonin in the last phase by the enzyme arylalkylamine N-acetyltransferase (AANAT), followed by the enzyme hydroxyanisole-O-methyltransferase (HIOMT). It is commonly believed that the rate-limiting step in the production of Melatonin is converting this acetylated form of serotonin, called acetylserotonin, to Melatonin [26,25]. melatonin free radical scavenging properties were identified 25 years ago [6]. After this discovery, Several in vitro [28,29] and in vivo [30,31] research that investigated Melatonin's capacity to suppress oxidative damage from harmful cells and tissues gave their approval to this discovery [32,33]. Highly reactive ROS can be stopped from harming molecules by either preventing the creation of their precursors, the weakly reactive ROS or by scavenging them as soon as they are produced. Melatonin has two primary mechanisms for protecting against molecular damage; direct ROS scavenger or radical avoidance, which involves lowering O2 production at the mitochondrial electron transport chain (ETC) [34,35,36,37]. Melatonin also encourages the enzymes that convert ROS into oxygen and water to prevent cellular damage. Glutathione peroxidases (GPx), superoxide dismutases (SOD1, SOD2), which eliminate oxygen, and peroxidases (GPx), which ride the cellular microenvironment of H2O2, are some of these enzymes [38,39]. At the same time, several clinical investigations have confirmed the effectiveness of Melatonin as a potent antioxidant [40,41,42]. Melatonin possesses another

advantage in that its product works as a scavenger of toxic species [43,44]. After interacting with a hazardous species, Melatonin undergoes an enzymatic or non-enzymatic transformation into additional antioxidants known as the "melatonin antioxidant cascade," which are just as effective at neutralizing free radicals as Melatonin [45]. Melatonin detoxifies several radical species, as opposed to the single radical scavenged by traditional antioxidants, through a series of processes [46].

Melatonin's Role in Vascular Alteration in AD

Other AD symptoms include neuronal malfunction, chronic inflammation, a decrease in the number of neurons [47], and aberrant changes in brain vascularization [48]. Vascular endothelial growth factor (VEGF) is a potential component that may be related to vascular changes in AD. VEGF participates in angiogenesis [49,50,51,52], vascular cell permeability [53,54,55], and neural development in the central nervous system [56]. The growing central nervous system exhibits VEGF and its two primary receptors, VEGFR1 and VEGFR2. Astrocytes secrete VEGF and interact with endothelial receptors to trigger downstream pathways that control angiogenesis [57]. There are at least five distinct VEGF variants in humans, including VEGF-121/145/165/189/206, each of which has a unique amino acid length [58]. The most prevalent isoform of VEGF-A, VEGF-165, is a pro-angiogenic isoform that participates in the process of angiogenesis [59]. VEGF contributes to the vascular pathology associated with AD; however, VEGF expression levels vary among studies.

There have been some studies that suggest VEGF may benefit AD models [60,61], But another study found that AD rats had higher levels of the angiogenic factor VEGF. According to the study by Puang et al., Melatonin may reduce the level of VEGF in the AD rat model's high expression [62]. Twenty-four mature male Sprague-Dawley rats were used in this study and separated into four groups. Melatonin plus A β administration has been given to one of these groups as a material to enhance the AD model. While in the other group, melatonin has administrated alone. In this study, the level of VEGF and the expression of its receptors were high in the cortex and hippocampus after the Aβ1–42 injection. Western blot assay and immunofluorescence staining were used to detect the level of VEGF in AD animals. Compared to the Aβ1–42-melatonin group., the expression of VEGF was dramatically reduced by melatonin administration alone in another component of VEGF, melatonin therapy alone

significantly reduced VEGFR1 expression In the cortex and hippocampus. Also, in VEGFR2, Melatonin showed a significant impact in reducing the expression of VEGFR2 in In the cortex and hippocampus [63]. These results confirm previous studies [64] that showed Melatonin's ability to downregulate VEGF.

Melatonin's Impact on the Cholinergic System

Cholinergic neurotransmission has been linked to several illness conditions. Since ACh plays a significant role in cognitive functions, the cholinergic system is highlighted as a contributing component in several types of dementia, including AD [65,66]. Any cholinergic transmission abnormality affecting the cortices and the hippocampi can cause cognitive and behavioural drawbacks [67]. The nucleus basalis of Meynert, a crucial source of cholinergic innervation for the cerebral cortex and hippocampus, undergoes a severe and particular deterioration in the AD brain [67,68]. Although choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities do not change until the later stages of the disease, acetylcholine levels decrease in the early stages of Alzheimer's disease [69,70]. Other scientific studies using tissue from autopsies and biopsies discovered a significant decline in ChAT activity in AD patients' neocortex, significantly correlated with dementia [71]. Even though the mechanism underlying the ACh shortage is yet unknown, the inhibitor of AChE has been utilized therapeutically. It is recognized as the gold standard of care for treating mild-to-moderate AD [72]. According to a former study, Melatonin has also been demonstrated to partially prevent the reduction of choline transport and ChAT activity in several neuronal proteins from synaptosomes and, more readily, from synaptic vesicles produced by peroxynitrite [73]. Furthermore, Melatonin could prevent neuropathological, behavioural, and biochemical alterations in eight-month-old APP695 transgenic mice with A deposition, substantial learning and memory problems, and a marked reduction in ChAT activity in the frontal cortex and hippocampus [74]. A different study showed that Melatonin might reduce ChAT activity in adult rats that had undergone ovariectomies [75]. Acetylcholine deficit can be treated with cholinesterase inhibitors, which can stabilize or stop changes in cognition, function, behaviour, and general [76]. Melatonin replacement therapy has been proven beneficial in treating sundowning, moderate cognitive impairment (MCI), an etiologically diverse condition that precedes dementia, and other sleep-wake disturbances in AD patients. Melatonin's potential as a therapy for AD is supported by its

ability to reduce AChE activity and scavenging reactive oxygen and nitrogen species, resolve sleep disturbances, and lower A toxicity [77]. No clinical studies compare Melatonin's efficacy versus the AChE inhibitor for AD patients. These two medications could work considerably better together, however. Tacrine-melatonin hybrids were recently created and synthesized as novel multifunctional therapeutic options for AD [78,79]. The combination has enhanced cholinergic and antioxidant activities, inhibiting human AChE more effectively and selectively than tacrine and scavenging free radicals more effectively than Melatonin. They have a common toxic effect and could be able to enter the central nervous system [80].

Melatonin's Impact on Beta-Amyloid and Tau Proteins

Amyloid refers to proteins that form elongated, unbranched fibrils in various cell types, including bacteria and mammalian tissues and organs. Such proteins are often categorized as amyloid if they are linked to illness and have been found in people's brains and other organs. It is known that several proteins may change into the amyloid aggregation form. These include fibrils linked to cancer, chronic degenerative disorders, and the typical physiological functions of animals and microbes. Unlike globular proteins, amyloidogenic proteins may aggregate into various distinctive amyloid forms, known as polymorphs. A particular polymorph can replicate itself by seeding other amyloid formations [81]. In the case of AD, brain tissues often contain amyloid fibrils made of one of two different proteins. These are tau and amyloid -peptide (Aβ). While Aβ fibrils are present in extracellular plaques, tau fibrils are discovered in intracellular tangles., and only fibrils of Aβ are seen in AD [82,83]. The positive role of Melatonin on the deleterious effects of Aβ has been shown in many ways, including as antioxidants have a positive impact on mitochondrial metabolism, antifibrillogenic and cytoskeletal, including the suppression of protein hyperphosphorylation [84,85].

Melatonin attempts to counteract the effects of Aβ were based on the initial discovery that the peptide causes oxidative stress, which results in mitochondrial DNA damage, protein carbonylation, lipid peroxidation, changes to membrane structure, changes to respiration, breakdown of the mitochondrial membrane potential, induction of antioxidant enzymes, and the production of heat-shock proteins [86,87,88,89]. Pappolla's research team's groundbreaking study was the first to offer strong evidence for effective neuroprotection against the toxicity of A in AD [90,91]. In actuality, melatonin treatment hindered the mortality of neuroblastoma

cells exposed to the Aβ peptide [92,93,94,95]. Melatonin's second anti-amyloid activity, fibrillogenesis, focuses on this process. Different methods have demonstrated that Melatonin inhibits the development of amyloid fibrils more effectively than other traditional antioxidants [96,97,98,99]. Indole-3-propionic acid, a melatonin structural analogue that also can be an effective radical scavenger, exhibits comparable or even stronger antifibrillogenic effects [100,101]. Using a transgenic mouse model, Melatonin was also found to reduce the development of amyloid plaque in vivo [102,103]. However, anti-amyloidogenic effects were not observed when the medication was begun in aged transgenic mice, after 14 months of life, despite the apparent histologically and behaviorally visible protection in these separate experiments [104]. That is to say, after a certain level of disease severity has been achieved, a drug like Melatonin is no longer capable of effectively inhibiting amyloid accumulation and amyloid-dependent damage. The third direction, the inhibition of protein hyperphosphorylation and the disruption of the cytoskeleton, has been chiefly researched in experimental systems that try to imitate the pathological alterations that characterize AD. Okadaic acid, a potent inhibitor of protein phosphatases one and 2A, led to cell death and mitochondrial malfunction in two lines of neuroblastoma cells [105,106,107]. Melatonin supplementation reduced lipid peroxidation, safeguarded cytoskeletal integrity, and stopped the okadaic acid-induced reduction in cell viability and mitochondrial metabolic activity [108,109]. In addition to reducing oxidative stress and tau hyperphosphorylation, Melatonin inhibits the phosphorylation system, particularly stress kinases. It reversed GSK-3 (glycogen synthase kinase 3)activation, demonstrating that its activities went beyond its antioxidant effects [110]. Neurotrophins and another tyrosine kinase (Trk) receptors, which constitute additional, crucial components of the phosphorylation system, were also demonstrated to be impacted by oxidants, including Aβ. Melatonin was able to restore normal trk and neurotrophin expression [111]. In another experiment, wortmannin [112] and isoproterenol [113] were used to cause tau hyperphosphorylation. Melatonin was observed to slow down the process of tau hyper-phosphorylation.

Conclusions

One of the most potent antioxidants is Melatonin, which functions on many levels, including direct radical scavenging, enzymatic control of oxidant generation, and mitochondrial radical

avoidance [114]. In terms of target cells and processes, Melatonin's pleiotropy is its most noticeable characteristic. It is not unexpected that Melatonin has been suggested as a therapy for AD and is protective in various experimental systems. Melatonin's anti-amyloid and indirect antioxidant properties are supported by proper circadian phasing and anti-excitotoxic activities [115,116]. This characteristic may partially prevent necrotic or apoptotic cell death, but it also has other mitochondrial actions that promote electron flow, proton potential, and ATP synthesis [117]. Early, long-term melatonin supplementation has been shown in prior studies employing APP transgenic mice to provide anti-amyloid and antioxidant advantages. However, this effect is eliminated when melatonin administration is started after the age of amyloid formation. [118,119,120,121]. Early and frequent melatonin supplementation may prevent AD or delay its onset. The inhibition of sundowning and promotion of sleep are important results supporting the use of Melatonin and the advantageous effects in experimental systems against cholinergic insufficiency, inflammation, fibrillogenesis, and tangle formation. Extensive clinical trials and studies using transgenic models are needed to verify the role of Melatonin in the late pathogenic stage of AD. If Melatonin has minimal effect in the later stages of the disease, research should concentrate on preventing AD rather than treating it.

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