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Low Intake of Methionine Increases the Risk Cartilage Damage of the Knee Joint through DNA Methylation of the IL-1β Gene and the Expression of IL-1 β

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ABSTRACT

Low intake of methionine lowers the quality of cartilage and chondrocytes modulate increases the secretion of protease and degeneration of cartilage. Exposure to low intake of methionine has great potential to decrease DNA methylation that can increase gene expression catabolic joint cartilage. The purpose of this study to prove a low intake of methionine (DL-methionine 0.00%) can decrease DNA methylation gene IL-1β and increase the risk of damage to the joint cartilage in rabbits *New Zealand (Oryctologus* cuniculus). Adult female rabbits were divided into 6 groups: three groups (1,2,3) normal rabbit and the next three groups (4.5 and 6) diincisi rabbit ACL. Formula DL-methionine were classified by the addition of DL -metionin per kg formula the addition of DL-methionine 0.25%; DL-methionine 0.15% and 0.00% DL- methionine. Rabbits will undergo acclimatization period of 7 days with standard polar food and randomly followed by administration of the formula DL-methionine for 35 days. Measurement of body weight once a week and every day eating left over measurements to calculate servings eaten per day per rabbit. After 5 weeks, the rabbits were sacrificed blood and tissue taken joint cartilage. Blood samples for examination of DNA methylation gene IL-1β methods and levels of serum IL-1βmethod *ELISA* using a commercial kit *RayBio* IL-1βRabbit. The tissue samples cartilage of the knee joint for examination of the expression of IL-1β immunohistochemical methods with the primary antibody anti-rabbit IL-1β using a commercial kit *Santa Cruz* (Sc7884) and damage to joint cartilage stained with H & E. The results of the study low intake of methionine (0.0% DL-methionine) can decrease DNA methylation gene IL-1β was significantly ($P < 0.05$), increased levels of IL-1 β serum ($p < 0.05$), and increased expression of IL-1 β ($p \leq 0.05$), and severity of knee joint cartilage. In conclusion low methionine intake has the potential of increasing the breakdown of joint cartilage through a reduction in DNA methylation gene IL-1 β and increased expression of IL-1 in the cartilage and the level of damage to the articular cartilage of the knee.

KEYWORDS: Low Methionine, DNA methylation gene IL-1β, destruction, joint cartilage

INTRODUCTION

When it is known that the pathogenesis of osteoarthritis significantly occurred because epigenetic changes, especially DNA methylation [3]. The cartilage of epigenetic changes cause changes in gene expression of cytokines, proteinase, extracellular matrix proteins [4,5,9,18]. Some research indicates there has been a deviation multiple gene expression catabolic such as IL-1β,*matrix metalloproteinases* (MMP), aggrecanase (ADAMTS-4, ADAMTS-5) and a number of other cytokines in cartilage of osteoarthritis [1,2, 19]. In epigenetic mechanisms of dietary factors have a strong and active effect changed the pattern of epigenetic at the end of modifying phenotypes [8]. Methionine is one of essential amino acids in protein synthesis is key, growth , normal development and a major source of methyl groups (CH3) and sulfur [24]. In the body methionine is converted to S-adenosy methionine (SAM) as the main donor for methyl endogenous DNA methylation. Methionine deficiency inhibit the formation of SAM causing the modification of DNA methylation and gene expression change.

In patients with osteoarthritis are known to have DNA catabolic genes such as IL-1β and gene expression of IL-1β gene in cartilage tissue increased [17, 11]. Interleukin -1β as major inflammatory mediators that can destroy joint cartilage, interleukin-1 β able to increase the secretion of the enzyme MMP-13 destruction cellular matrix molecules especially type II collagen and decrease the chondrocytes cell synthesize proteoglycans cartilage damage.

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MATERIALS AND METHODS

Animals Model

Female, white, 4-6 months old, weigh around 1500-2500 gram, *New Zealand* rabbits (Oryctologus cuniculuc), were used as an animal model. The rabbits were acquired from Modern Rabbit Farming under Batu Animal Husbandry Department. To accelerate joint cartilage damage OA used incision modeling on the anterior cruciate ligament (ACL). ACL incision procedure refers to a method Hulth modified by the procedure of Balai Besar Pelatihan Peternakan Animal Clinic, Batu, East Java. All care and experimental procedures carried out by the study protocol approved by the Ethics Committee for Health Research, Faculty of Medicine, University of Brawijaya based on the number 372 / EC / KEPK / 09/2016.

Study design

Research design was using experiment. Rabbits divided into six treatment groups, namely Group (1) normal rabbit got the formula DL-methionine 0.25%; group (2) normal rabbit got the formula 0.15% The DL-methionine; group (3) normal rabbit got the formula DL-methionine 0.00%. Then the group (4) rabbit ACTL got the formula DL-methionine 0:25%; group (5) ACTL rabbit gets the formula DL-methionine 0.15% and the group (6) rabbit ACL got the formula DL-methionine 0.0%. Giving formula DL-methionine for 5 weeks and got extra green vegetables as much as 30 g / day / head. Methionine formula feeding conducted in the morning at 07.00 and greens given afternoon at 15.00.

Methionine formula

Low methionine Formula prepared in the Laboratory of Food Technology Nutrition Department of Ministry of Health Polytechnic of Malang. Three formulas methionine is formulated using a mixture of local food with protein, fat and carbohydrates the same, namely 12.86% protein; fat 8.7%, carbohydrates 65.41%. Methionine is used in the form of DL-methionine and the amount added to the formula methionine different (ie 0, 25% DL-methionine (methionine enough)_control formula; 0, 15% DL-methionine (methionine medium) and 0% DL-methionine (lowmethionine). Composition Formula methionine (g / 1000g) as in table 1.

Table 1. Composition of Formula methionine

No	Food ingredients	Formula methionine (per 1000 g)		
		DL-methionine (0.25%)	DL-Methionine (0.15%)	DL-Methionine (0.00%)
1	Corn Starch (g)	600.00	600.00	600.00
2	Bran (g)	100.00	100.00	100.00
3	Polar (g)	100.00	100.00	100.00
4	soy flour (g)	100.00	100.00	100.00
5	Oilseeds (g)	82.0	82.0	82.0
6	Salt (g)	5	5	5
7	Vitamins (g)	2	2	2
7	Mineral (g)	1	1	1
8	DCP (g)	10	10	10
9	DL- Methionine (g)	2.5	1.5	0.00

Blood Collection bunny

Rabbit blood sampling performed on veins *auricular* much as 4*cc,* through a large vein in the ear aseptic event. Then the blood is inserted into *vacutainer* without coagulant (closed warn a red) by 2 cc and *vacutainer* with anti coagulant EDTA (purple cap) of 2 cc.

Body Weight Measurement

Weight measurement rabbit individually performed once a week using electric scale sbrand *CAMCRYACS 15-JC33*type,capacity of 30 kg with a precision of 1 g. Weighing done in the morning before the administration of low methionine formula. Weight gain is calculated by subtracting the results of the weighing of the end of the initial weight expressed in g.

Intake measurement formula methionine

Methionine formula intake was measured by weighing the food remains methionine formula every day for 35 days using scales. *a triple beam* Formula intake of methionine calculated by subtracting the amount of formula given by the number of residual formula is not consumed. The average intake of methionine per day calculated by dividing the total intake of methionine divided 35 day formula and expressed in g per day.

Serum IL-1β measurement

Elisa methode was employed to measure serum IL-1β and performed according to ELISA commercial kit instruction Ray Bio Rabbit IL-1 β, ELL –IL-1β). A 50 µl blanko and standard solution were put pipetted into

empty wells. A total of 50 µl of blood serum each sample and put into the wells and incubated in at 37 °C, covered with thin-foil wrap for 30 minutes. The solution was rinsed 4 times with PBST ,added 50 µl, secondary antibody conjugated with HRP and incubated once more in 37 ° C, covered with thin wrap, for 30 minutes. After that, the solution was rinsed 4 times with PBST and added 50µl tetramethylbenzidine (TMB) was added in a dark room. After 15 minutes of 37 ° C incubation stop solution (in NaOH) was added and the ELISA plate was read at 450 nm wavelength.

Examination of DNA methylation of gene promoters of IL-1β

Based on the analysis of Bioinformatics NCBI, DNA sequencing IL-1β gene is located on chromosome 2 rabbits at the site of the reverse strand and position 97.614851-67.618656 3589-3846 9. The results of DNA sequencing promoter region *geneof IL-1 β* has a length of 285 bp the sequence of the primary design F: TAA GGA GGA ATT GTT GTT TGT TGA T and R: CTC ACT CTT AAT AAA TTT AAA CCC A, Left primer 3589 25 58.94 52.00 5 GATAAGTTGGAATTTGAGTTTGTT, primary Right 3846 25 59.80 44.00 4 AAAACTCTCCTTAATTTTCCCAAAA. Whole-genome DNA isolation results do CT *conversion* by using *the EZ DNA methylation GOLD "Kit* (Catalog Nos.D5005 & D5006) with procedures the protocols listed in the Kit. For PCR mix using KOD-Multi & epidemic Toyobo (catalog F1440K). DNA Sequencing of IL-1 gene is carried out by PCR using Sequence Scanner 2 of Thermo Fisher. Data obtained by equated to see the similarities and differences in the nucleotide sequence of each group and DNA methylation levels of IL-1 β are calculated.

Examination of the expression of IL-1β

Expression of IL-1β in the cartilage tissue of a rabbit knee joints examined Immunohistochemistry with primary antibody rabbit polyclonal anti-IL-1β using a kit *Santa Cruz* (Sc7884). The procedures performed following the protocol of the manufacturer modified, beginning by *blocking endogenous* peroxidase, *blocking* protein un spesifik buffer *(backgroun sniper).* Then the primary anti-rabbit polyclonal anti-rabbit antibody IL-1 β, incubating overnight at 4 ° C. After it was allowed to reach room temperature and washed with BPS 3 x 5 minutes, incubated with secondary antibody *(biotin Conjugate)* for 60 minutes, washed with PBS 3x5 minutes and added to slide with DAB (DAB chromagen: DAB buffer = 1:40), incubated at room temperature for 1-10 minutes and washed with 3x5 aquade minutes. Furthermore added to the slide with solution *Mayer'shematoxilen* (Mayer's *Hematoxilen: Distilled water* = 1: 3) and incubation at room temperature for 1-10 minutes, then Mouting with etellan. Observations chondrocyte cells that express IL-1 β under the microscope O*lympus* B x 51 and 400 times magnification objective.

Level of Cartilage Damage of Knee Joint

Damage to cartilage of the knee joint is assessed from the presence of fissure on the surface of the knee joint cartilage with H & E staining and the proteoglycan content demonstrated from the safranin-O staining intensity. Assessment of cartilage damage of the knee joint with using Mankin's scoring system modification with give score in each sub category, the total of the assessment results of each sub category shows the level of damage of joint cartilage.

RESULTS

Body Weight Animals

Having given formula DL-methionine for 35 days, appeared to be changes in body weight as shown in the chart rabbit weight development rabbit in figure 1

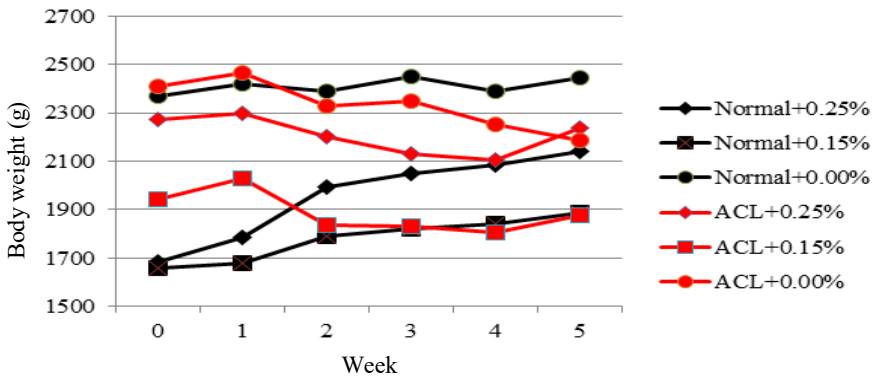

Figure 1. The development of body weight (g/week)

On The first week adaptation phase seemed no weight changes, enters week two begin no weight change. Normal rabbit group body weight tends to rise, the lowest weight gain of about 2.74% in rabbits given formula DL-methionine 0.00%. While the rabbit ACL body weight development tends to fall with the highest weight loss of 7.99% in the rabbit ACL by formula DL-methionine 0.00%. Based on the results test *of one-way ANOVA* against weight gain in normal rabbit group there is a significant difference with $p = 0.024$ ($p \leq 0.05$), and showed no difference with $p = 0677$ ($p > 0.05$) in the group of rabbits ACL. Additions and weight loss associated with the intake of formula rabbit DL-methionine and activity. Figure 2 shows the average intake of food formula DL-methionine per day.

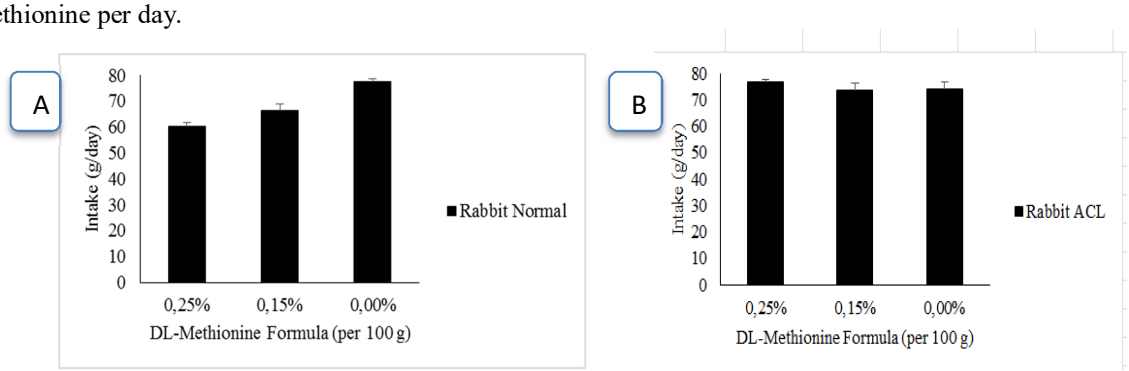

Figure 2. Intake of Food Formula DL-Metionin per day

Serum IL-1β level

Serum IL-1β levels in the normal rabbit group were significantly different between groups with significant values of $p = 0.000$ ($p \leq 0.05$). Administration of a low-methionine formula (DL-methionine 0.00% formula) in normal rabbits had higher levels than rabbits given the formula of DL-methionine 0.25%. In the ACL rabbit group there was a difference in IL-1β levels between rabbit groups with indigo $p = 0.04$ (<0.05). Figure 3. shows the levels of IL-1 beta serum of normal rabbits and ACL rabbits.

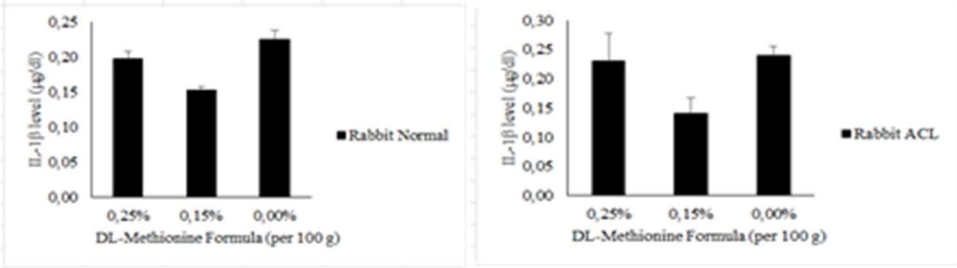

Figure 3. Serum IL-1 level

Methylation DNA gen IL-1β

CpG Based on the analysis of gene IL-1 β CpG in the promoter region *island* (258bp) gene lies only 7 sites IL-1β is the site 25, 48, 81, 116.118, 142, and 153. in normal rabbits and rabbit ACL positioned IL-1β gene in the promoter region of IL-1β gene together, it shows that the incision ACL does not cause changes in methylation patterns IL-1β gene DNA. In the figure 3 shows the position of IL-1β gene in the promoter region of IL-1β gene and gene methylation status of IL-1 β

In rabbits given formula DL-methionine 0.00%, there are 3 sites of gene IL-1β which do not undergo methylation that sites 25, 81 and 153, as indicated by the change in base citosin Guanine (CG) to thymine Guanine (TG) in the DNA sequence in IL_1β gene promoter region. While the group of rabbits given formula DL-methionine contained 0.15% The 25 sites which do not undergo methylation, whereas by the formula DL-methionine 0:25% of seven (7) sites in the gene IL-1β gene promoter region of IL-1 β methylated all. The effect of low intake of methionine on the methylation status as shown in Figure 4.

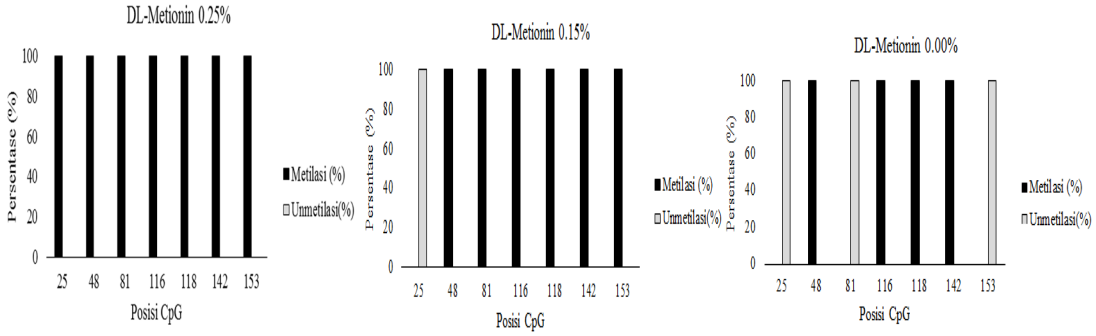

Figure 4. A low intake of methionine induces a decrease in IL-1 β rabbit gene methylation

Figure 4, shows that a low methionine formula (Dl-methionine 0.00%) has an effect decrease IL-1β gene methylation or increase unmetilation of IL-1β gene, thus enhancing IL-1β expression. Kruskal Wallis test showed that there was significant difference $p \leq 0.005$ to IL-1 β methylation between rabbit groups

Expression of IL-1 β in Cartilage Knee Joints

Interleukin -1 β (IL-1β) is a local inflammatory mediators have an important role in the destruction of joint cartilage. Based on the calculation of the average expression of IL-1 β in the knee joint cartilage of rabbits are presented in figure 5

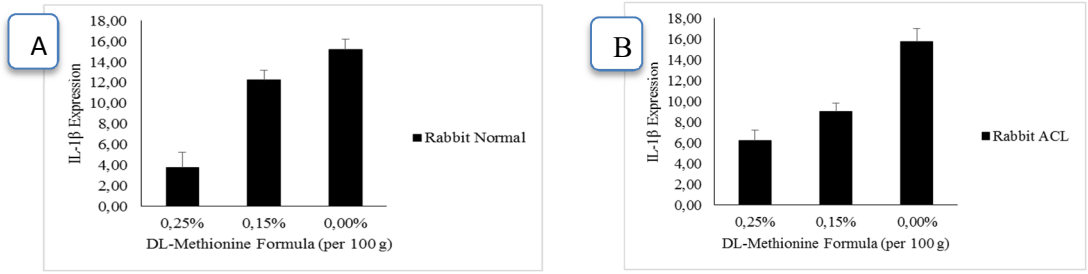

Figure 5. IL-1β expression in cartilage joints of the knee joint (A) normal rabbit group, (B) group of ACL rabbits

The results of the one-way ANOVA test on the expression of IL-1β in normal rabbit group significant difference with significant p value $\leq 0.05 = 0.01$. In the rabbit ACL group of one-way ANOVA test results, there were significant differences with significant value ≤ 0.05 $p = 0.00$. Results of examination with immunohistokima expression of IL-1β in cartilage tissue in figure 6.

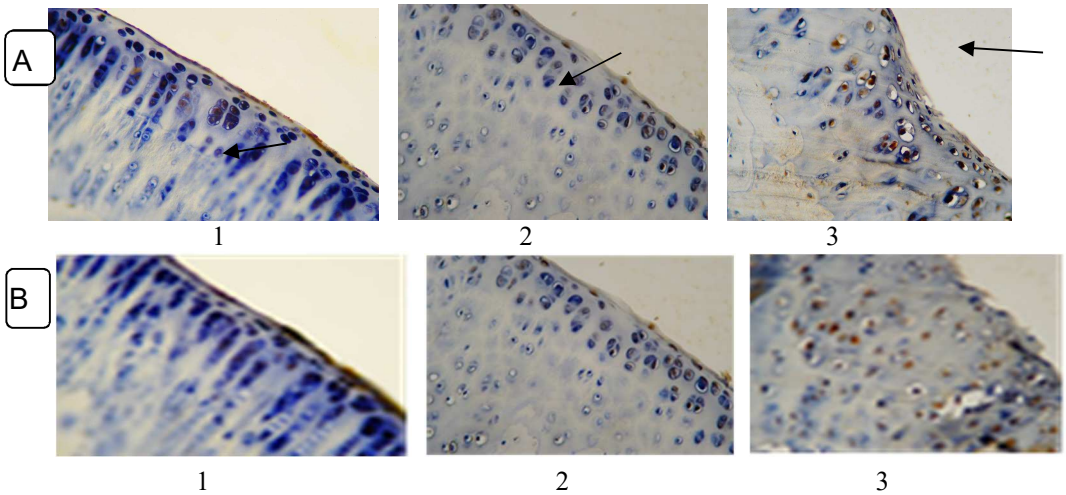

Figure 6. Expression of IL-1β by immunohistochemical methods (magnification 400 x) looks brown in the cytoplasm of cells.

Description:
A. The preparation of joint cartilage normal rabbit knee joint cartilage
B. Preparations knee incision ACL
1. Rabbit + Formula DL-methionine 0.25%
2. Rabbit + Formula DL-methionine 0.15%
3. Rabbit + Formula DL -metionin 0.00%

Cartilage Damage knee joint

Total average score of cartilage damage assessment of the rabbit knee joint as presented in figure 7

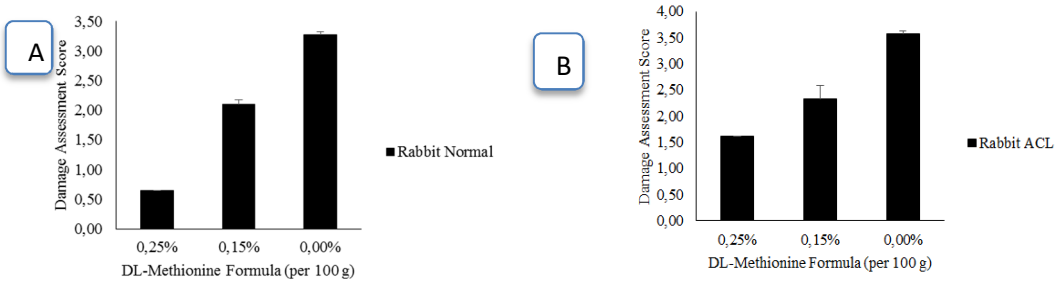

Figure 7. Score of of cartilage damage (A) normal rabbit group, (B) group of ACL rabbits

Based on the test *Kruskal-Wallis* showed that the obtained value of p = 0.006 less than 0.05, it can be concluded least there were no differences between groups, followed by test. Histology of cartilage damage based on changes in the structure of cartilage that rated the fissures in the surface layer joints with H & E staining and staining with safrani-0 to evacuate glycosaminoglycan content judged from the intensity of the illumination. Histology of cartilage structure as shown in Figure 8 and picture lighting intensity as in Figure 9.

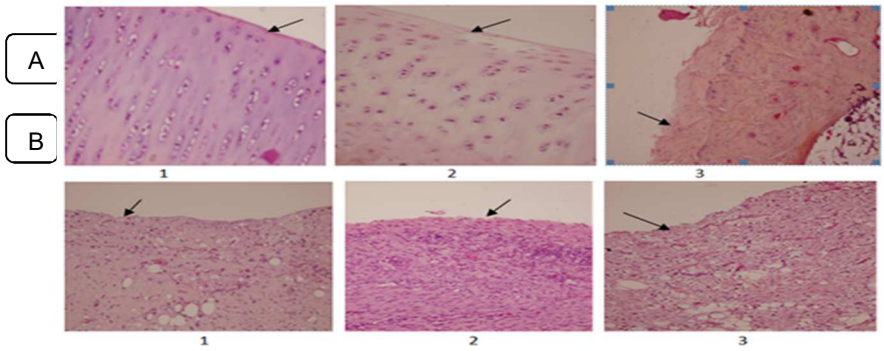

Figure 8. Histology of the rabbit knee joint cartilage

Description:
A. The preparation of joint cartilage normal rabbit knee joint cartilage
B. Preparations knee incision ACL
1. Rabbit + Formula DL-metionin 0.25%
2. Rabbit + Formula DL-metionin 0.15%
3. Rabbit + Formula DL- methionine 0.00%

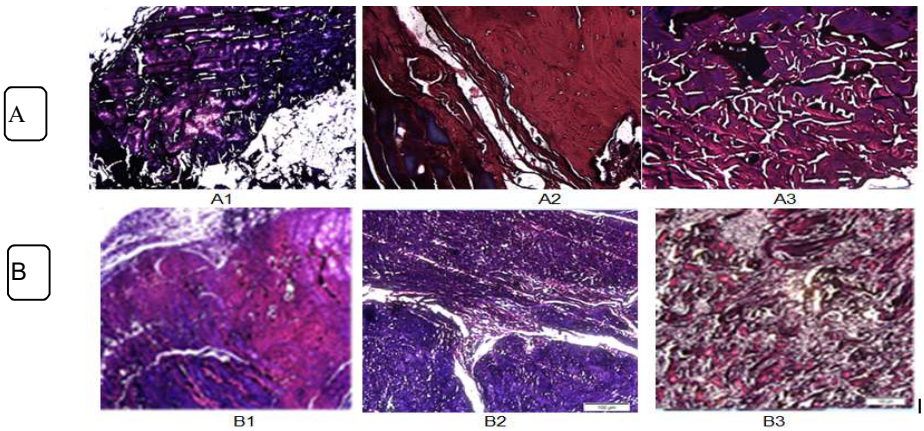

Figure 9. Safranin O staining intensity in joint cartilage

Description:
A. The preparation of joint cartilage normal rabbit knee joint cartilage B. Preparations knee incision ACL
1. Group by giving formula 0:25% DL- methionine (control)
2. Group with a formula giving methionine DL-methionine 0.15% the
group with the provision of the formula 3. methionine 0.00% DL-methionine

DISCUSSION

In this study the formula giving low methionine (DL-methionine 0.00%) in normal rabbit 35 days can increase the weight of about 65.0 g (2.74%) was lower than in rabbits given formula DL- methionine 0.25% reaching 375 g. Similar results were observed in mice fed a diet low in methionine (DL-methionine 0.00%) during the 33-day weight gain is lower than the control group to 38% [21]. Other studies have diets low in methionine administration in rats for 3 months of weight gain of 45% was slightly lower than the control group [7]. Methionine restriction in mice can reduce fat mass, improve insulin sensitivity and affect fat metabolism, energy and health status [14, 15, 20]. In normal conditions the restriction of methionine to maintain body weight remains constant by increasing the use of energy, involving an increase in energy expenditure [13].

Levels of Interleukin-Iβ serum

In this study, administration of DL-methionine formula different in normal rabbits causes a difference to the levels of serum IL-1β was significantly ($p = 0.008$) and no difference ($p = 0.064$) in the group of rabbits ACL. Giving formula DL-methionine 0.25% in normal rabbit group is able to inhibit the increase of serum IL-1β and giving formula DL-methionine 0.00% may increase serum levels of IL-1β and thus potentially have an effect on low-level inflammation.

Interleukin -1 (IL-1β) is a major inflammatory cytokine that plays an important role in the infection process and aging-related diseases as well as the innate immune response. In older people IL-1β play a role in normal homeostasis and as an inflammatory response is responsible for the development of chronic disease [6]. As a major inflammatory cytokines interleukin -1 β (IL-1β) have autocrine and paracrine effect, so that the serum levels of IL-1β is often used as an index of non-specific inflammation. Physiology of food intake involved in the inflammatory response and oxidative stress [10], changes in plasma amino acid concentrations causing malnutrition and inflammatory conditions

DNA Methylation

DNA methylation becomes one of the epigenetic modifications which is the reaction of adding methyl groups (CH3) to the carbon position of 5 'cytosine and producing the silencing gene phenotype.This addition of methyl (CH 3) groups in C causes chemical structure changes that can not be attached by transcription factors binding to the promoter's DNA causing the inactivation of genes (silencing genes). This DNA methylation process is mediated by the DNMT 1 enzyme and DNMT1 activity is affected by the level of S-adenosylmethionine (SAM) [4]. Methionine as an essential amino acid derived from food can contribute about 60% of exogenous methylsatures to S-adenosylmethionine (SAM) substrates and important DNMT1 enzyme activity in the methylation process.

In this research, the provision of DL-methionine 0.00% causes the availability of SAM methyl (CH3) endogenous sources and the activity of DNMT decreases. In this study, rabbits who were given DL-methionine 0.00% formula contained 3 un-methylated CpG IL-1β sites.Several studies that have been done on OA patients show that there is a loss of DNA methylation in the CpG IL-1β promoter region that will express the IL-1β gene [22]. DNA demetilation in the long-acting IL-1β gene promoter region causes a deviation of persistent gene expression [11,12,16].

Expression -1β

Under normal conditions IL-1β plays a role in maintaining homeostasis and as an inflammatory response responsible for the development of chronic disease that occurs in the elderly [6]. Bonds between IL-1β receptors and IL-1β ligand are able to alter condyting phenotypes to more catabolic condrosites [2]. In this study normal rabbits were given a low-methionine formulation (DL-methionine 0.00%) having a 5-fold higher-than-normal 5-fold IL-1β expression compared to a normal rabbit given a 0.25% DL-methionine formula.

In ACL rabbits IL-1β gene expression is higher than normal rabbit, but has a DNA methylation status of IL-1β gene same as normal rabbit.Several previous studies have shown that ACL incision in rabbits causes condylits to synthesize IL-1β at higher levels in post-trauma [6,11]. Other studies have shown that acute ACAR acute incarceration in experimental animals leads to biochemical, biomedical changes and provides a higher release of inflammatory mediators [1,6,10]. In a study using human subjects, after a knee injury occurs a metabolic imbalance and biomechanical changes that aggravate cartilage damage and cause osteoarthritis [2,4].

Cartilage Damage Knee Joint

In this study a low intake of methionine (a formula DL-metionin 0.00%) can be increases the risk of cartilage damage of the knee joint through decreased methylation of the Il-1β gene DNA. A low intake of methionine causes the availability of methionine in the body is reduced resulting in a decrease in the formation of S-adenosylmethionin (SAM) and the activity of the enzyme DNMT1.

Decreased DNA methylation of the IL-1β gene induces expressed IL-1β which is a major inflammatory mediator in cartilage damage. Increased expression of IL-1β in the joint cartilage tissue causes cell chondrocytes produce MMP-13 high and inhibit proteoglycan synthesis. MMP-13 is a degrading enzyme extracellular components matrix joint cartilage especially collagen type II. In this study increased intake of methionine can inhibits decreased DNA methylation of IL-1β gene, all CpG sites of the IL-1β gene on the promoter region of the IL-1β-termylated and IL-1β genes were low in the rabbit group given the DL-methionine 0.25% formula [23,24].

CONCLUSION

This study showed that a low intake of methionine (DL-methionine 0.00%) may cause low-level inflammation, and increase joint cartilage damage through decreased DNA methylation of IL-1β gene, increased IL-1 β expression in joint cartilage tissue . Further research is needed to determine the effect of low intake of methionine on the expression of TGF-β as an anabolic gene cytokine in cartilage tissue.

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Numerical Analysis of a Delayed SIR Epidemic Model with Constant Vaccination Effect

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ABSTRACT

In this work, a vaccinated delayed SIR (Susceptible, Infected, and Recovered) epidemic model with bilinear incidence rate and susceptibility effect for the transmission dynamics of an infectious disease in human population has been constructed and analyzed both dynamically and numerically. An unconditionally convergent numerical model i.e. Non Standard Finite Difference (NSFD) scheme has been developed by aiming that the continuous and the discrete models must exhibit the same behavior. Well-known numerical schemes like Forward Euler's method and RK-4 method are used to compare the results. It is shown that both methods are failed for large time step and/or delay factor 'τ'. Whereas, for all time steps and delay factors 'τ', the proposed numerical model preserves structure of the continuous dynamical system. Moreover, the effectiveness of vaccination has also been presented on different vaccination coverage levels for both Susceptible and Infected compartments.
KEYWORDS: Epidemic model, Delay factor, Constant vaccination, Numerical analysis, Convergence

1. INTRODUCTION

Controlling of infectious diseases is a burning issue of current era. Vaccination is a key tool and tactic to control the spread and the eradication of an infectious disease [1-4]. For example, in the case of measles, it is advised to vaccinate all infants up to the age of 15 months and then revaccinate approximately after 6 years to increase the immunization. Such type of recommendation is known as constant vaccination strategy [5]. Some childhood diseases like measles, chicken pox, mumps, etc. can also be prevented by using constant vaccination strategy [6]. Many time-delay epidemic models with vaccination strategy are present in history as in [7-11]. In 2008, Buonomo *et al.* [9] studied an SIR epidemic model with information dependent vaccination. In the following Delayed SIR epidemic model, the effect of constant vaccination is added with the assumptions that the birth rate 'A' and the death rate μ are not equal, the effectivity of the vaccination is 100% and 'p' percent of the vaccinated susceptible population will prevent the 'p' percent population from disease.

Mathematical models of the transmission dynamics of infectious diseases are systems of coupled non-linear ordinary differential equations [6, 12-15]. These systems are very hard to solve analytically. Some authors used semi analytic techniques to solve these epidemic models. A detailed description for these methods is given in [16] and the references there in. A reliable numerical investigation is needed when we have to deal with a non-linear system of differential equations. Most of the semi analytic and standard numerical techniques do not exhibit the true behavior of the mathematical models in population dynamics. These techniques fail to preserve some essential properties like positivity of the solution, boundedness of the sub population, and true steady states of the continuous models.

In this work, a competitive numerical scheme is proposed to solve a system of non-linear coupled differential equations with the effect of time-delay. Analysis of proposed scheme proves its unconditional convergence and dynamical consistency with the continuous model.

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2. FLOW CHART OF VACCINATED SIR MODEL

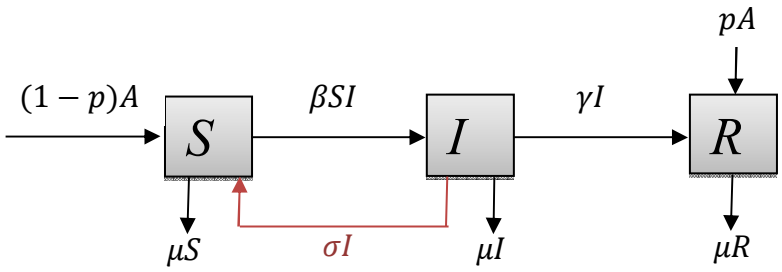

Fig. 1. Flow diagram of Vaccination Model

where,
S = Susceptible individuals
I = Infected individuals
R = Recovered individuals
A = Population recruitment rate
p = Vaccination rate
μ = Death rate
β = Rate of conversion from susceptible to infected individuals
γ = Rate of conversion from infected to recovered individuals
σ = Rate of conversion from infected to susceptible individuals

2.1 System of Differential Equations

The differential equations obtained from above model (Fig.1) are;

$$\begin{aligned}\frac{dS}{dt} &= (1-p)A - \mu S - \beta SI + \sigma I \\ \frac{dI}{dt} &= \beta S(t-\tau)I(t-\tau)e^{-\mu\tau} - (\sigma+\mu+\gamma)I \\ \frac{dR}{dt} &= \gamma I - \mu R + pA\end{aligned} \qquad (1)$$

where, $\tau \geq 0$ is an incubation period. This is a time through which an infected individual will become infectious i.e. it can spread the infection further [17, 18, 19]. The incidence rate $\beta S(t-\tau)I(t-\tau)e^{-\mu\tau}$ appearing in the second equation of system (3) represents the rate at time $t-\tau$ at which susceptible individuals are leaving the susceptible class and entering the infectious class at time t [20].

Here $S + I + R = N$

and $\frac{dS}{dt} + \frac{dI}{dt} + \frac{dR}{dt} = (1-p)A - \mu(S+I+R) + pA = A - \mu = 0$;

because rate of change of sub populations is constant.

It is enough to consider the first two equations of above system (1) as these are independent of R. Hence system (1) becomes

$$\begin{aligned}\frac{dS}{dt} &= (1-p)A - \mu S - \beta SI + \sigma I \\ \frac{dI}{dt} &= \beta S(t-\tau)I(t-\tau)e^{-\mu\tau} - (\sigma+\mu+\gamma)I\end{aligned} \qquad (2)$$

3. CONDITION FOR EPIDEMIC

$R_0 = \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu(\sigma+\mu+\gamma)}$ is a **threshold parameter** or **basic reproductive number** of the proposed model. It plays a vital role to control a disease by switching it from one equilibrium point to the other.

3.1 Equilibrium Points of the Model

The two equilibrium points of the epidemic model are $E_1 = (S, I) = \left(\frac{(1-p)A}{\mu}, 0\right)$, Disease Free Equilibrium (DFE) if $R_0 < 1$ and $E_2 = (S^*, I^*) = \left(\frac{(1-p)A}{\mu R_0}, \frac{(1-p)A\mu(1-R_0)}{\mu\sigma R_0 - \beta(1-p)A}\right)$, Endemic Equilibrium (EE) if $R_0 > 1$.

3.2 Local Stability of DFE Equilibrium

To analyse the local stability of the DFE point i.e. $E_1\left(\frac{(1-p)A}{\mu}, 0\right)$,

let $f = \frac{dS}{dt} = (1-p)A - \mu S - \beta SI + \sigma I$

and $g = \frac{dI}{dt} = \beta SIe^{-\mu\tau} - (\sigma + \mu + \gamma)I.$

Differentiating f and g partially with respect to S and I, we get,

$f_s = -\mu - \beta I$

$f_I = -\beta S + \sigma$

$g_s = \beta Ie^{-\mu\tau}$

$g_I = \beta Se^{-\mu\tau} - (\sigma + \mu + \gamma)$

At $E_1\left(\frac{(1-p)A}{\mu}, 0\right)$, all above partial derivatives become,

$f_s = -\mu$

$f_I = -\frac{\beta(1-p)A}{\mu} + \sigma$

$g_s = 0$

$g_I = \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu} - (\sigma + \mu + \gamma)$

Jacobian matrix of the system (2) is defined as,

$$J = \begin{pmatrix} f_s & f_I \\ g_s & g_I \end{pmatrix} = \begin{pmatrix} -\mu & -\frac{\beta(1-p)A}{\mu} + \sigma \\ 0 & \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu} - (\sigma + \mu + \gamma) \end{pmatrix}$$

From this Jacobian matrix we obtain the following eigen-values,

$$\lambda_1 = -\mu \quad \text{and} \quad \lambda_2 = \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu} - (\sigma + \mu + \gamma)$$

Clearly $\lambda_1 < 0$, but we need to prove it for λ_2.

Let $\lambda_2 < 0$

$\Rightarrow \quad \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu} - (\sigma + \mu + \gamma) < 0$

$\Rightarrow \quad \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu} < (\sigma + \mu + \gamma)$

$\Rightarrow \quad \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu(\sigma+\mu+\gamma)} < 1$

$\Rightarrow \quad R_0 < 1$, where $R_0 = \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu(\sigma+\mu+\gamma)}$

Which means $\lambda_2 < 0$ if $R_0 < 1$.

Hence the DFE point is locally asymptotically stable if $R_0 < 1$.

4. NUMERICAL ANALYSIS

A competitive numerical scheme based on non-standard finite difference (NSFD) modelling proposed by R.E. Mickens [21, 22] is presented in this section.

4.1 NSFD Method

Let S^n and I^n denotes the values of $S(t)$ and $I(t)$ at $t = n$. Using non-standard finite difference modeling theory, System (2) can be written as follows;

$$S^{n+1} = \frac{S^n + \varphi(h)((1-p)A + \sigma I^n)}{1 + \varphi(h)(\mu + \beta I^n)}$$

$$I^{n+1} = \frac{I^n + \varphi(h)\,\beta S^{n+1} I^n e^{-\mu\tau}}{1 + \varphi(h)(\sigma + \mu + \gamma)} \qquad (3)$$

Where $\varphi(h)$ is a function such that $\varphi(h) \to 0$ as $h \to 0$.

4.2 Convergence Analysis

In this section, the stability analysis of the DFE point i.e. $E_1 = (S, I) = \left(\frac{(1-p)A}{\mu}, 0\right)$ is performed for the proposed NSFD method.

By considering equations of system (3);

let $f = S^{n+1} = \frac{S + \varphi(h)(A(1-p) + \sigma I)}{1 + \varphi(h)(\mu + \beta I)}$

and $g = I^{n+1} = \frac{I + \varphi(h)\,\beta SIe^{-\mu\tau}}{1 + \varphi(h)(\sigma + \mu + \gamma)}$.

$$\text{Jacobian Matrix} = J = \begin{pmatrix} f_S & f_I \\ g_S & g_I \end{pmatrix}$$
$$= \begin{pmatrix} \frac{1}{1+\mu\,\varphi(h)} & \frac{\sigma\varphi(h)[1+\mu\,\varphi(h)\,]-\beta\varphi(h)\left[\frac{(1-p)A}{\mu}+(1-p)A\,\varphi(h)\right]}{[1+\mu\,\varphi(h)]^2} \\ 0 & \frac{\mu+\varphi(h)\,\beta\,(1-p)A\,e^{-\mu\tau}}{\mu\,[1+\varphi(h)(\sigma+\mu+\gamma)]} \end{pmatrix}$$

From above Jacobian matrix we obtain the following eigen-values,

$$\chi_1 = \frac{1}{1+\mu\,\varphi(h)} \quad \text{and} \quad \chi_2 = \frac{\mu+\varphi(h)\,\beta\,(1-p)A\,e^{-\mu\tau}}{\mu\,[1+\varphi(h)(\sigma+\mu+\gamma)]}$$

Clearly, $\chi_1 < 1$, but we need to prove it for χ_2

let $\chi_2 < 1$

$$\Rightarrow \quad \frac{\mu+\varphi(h)\,\beta\,(1-p)A\,e^{-\mu\tau}}{\mu\,[1+\varphi(h)\,(\sigma+\mu+\gamma)]} < 1$$
$$\Rightarrow \quad \mu + \varphi(h)\beta(1-p)A\,e^{-\mu\tau} < \mu[1+\varphi(h)\,(\sigma+\mu+\gamma)]$$
$$\Rightarrow \quad \varphi(h)\,\beta(1-p)Ae^{-\mu\tau} < \mu\varphi(h)(\sigma+\mu+\gamma)$$
$$\Rightarrow \quad \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu(\sigma+\mu+\gamma)} < 1$$
$$\Rightarrow \quad R_0 < 1, \text{ where } R_0 = \frac{\beta(1-p)Ae^{-\mu\tau}}{\mu(\sigma+\mu+\gamma)}$$

Which means $\chi_2 < 1$.

Hence the DFE point is locally asymptotically stable for the proposed NSFD scheme of Vaccinated Delayed SIR model, which means that the proposed scheme converges to DFE point for any arbitrary value of time step 'h' whenever $R_0 < 1$.

5. NUMERICAL EXPERIMENTS

Numerical experiments are performed by using the parameter values given in the table 5.1.

Table 5.1: Table of parameter values

Parameters	DFE	EE
δ	0.1	0.1
A	0.95	0.95
μ	0.05	0.05
β	0.01	0.1
γ	0.5	0.5
τ	≥ 0	≥ 0
p	[0,1]	[0,1]

5.1 Comparison of Methods (Numerical schemes)

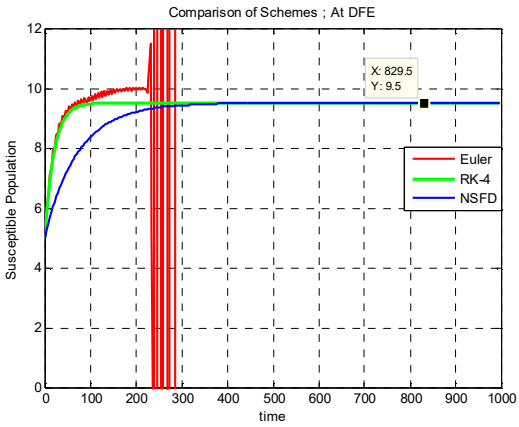

Figure 2: For $h = 3.5$, $\tau = 7$ and $\boldsymbol{p = 0.5}$, at DFE

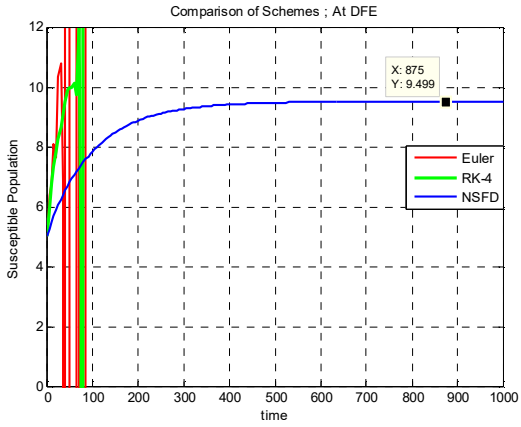

Figure 3: For $h = 5$, $\tau = 10$ and $\boldsymbol{p = 0.5}$, at DFE

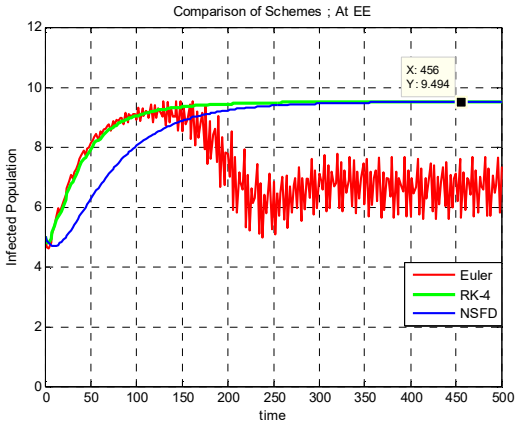

Figure 4: For $h = 2$, $\tau = 12$ and $\boldsymbol{p = 0.5}$ at EE

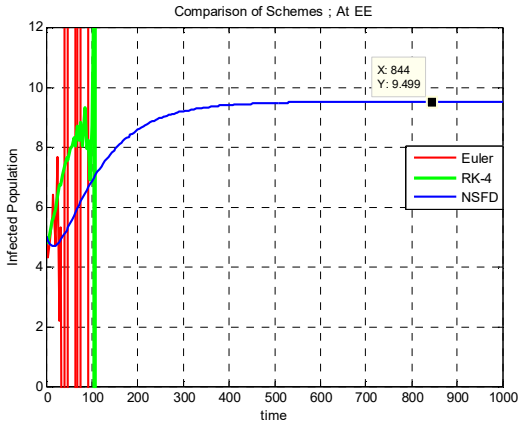

Figure 5: For $h = 4$, $\tau = 12$ and $\boldsymbol{p = 0.5}$ at EE

5.2 Effect of Delay factor τ by using NSFD Method

The effect of τ has been studies at different levels of vaccination.

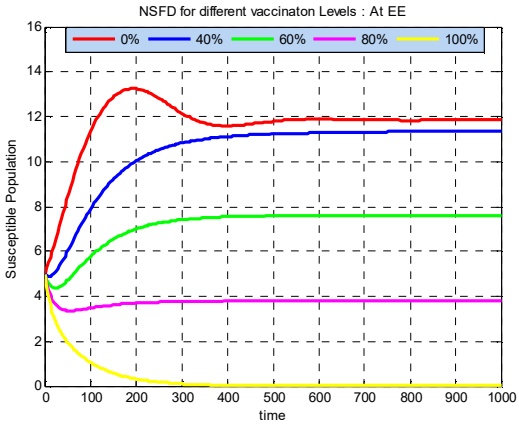

Figure 6: Susceptible population for $h = 4$ and $\tau = 12$ at EE

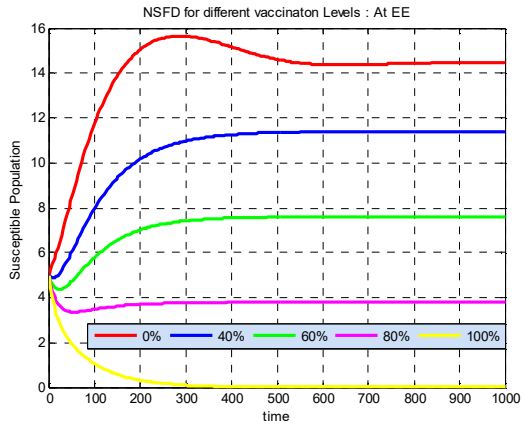

Figure 7: Susceptible population for $h = 4$ and $\tau = 16$ at EE

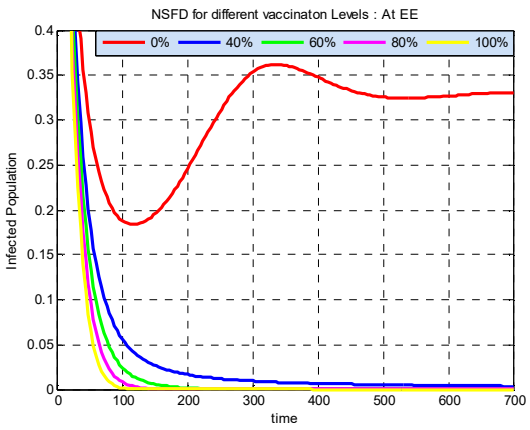

Figure 8: Infected population for $h = 4$ and $\tau = 12$ at EE

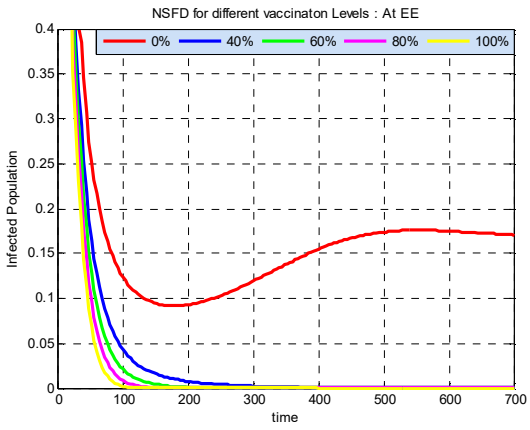

Figure 9: Infected population for $h = 4$ and $\tau = 16$ at EE

6. RESULTS AND DISCUSSION

The transmission dynamics of some disease with Vaccinated Delayed SIR model has been analysed dynamically and numerically. An un-conditionally convergent non-standard finite difference (NSFD) numerical model is proposed and convergence analysis of DFE point is presented. Numerical experiments are performed for different values of discretization parameter 'h', time-delay 'τ' and vaccination coverage levels 'p'. From figure 2, 3, 4 and 5, the comparison of proposed NSFD scheme with Forward Euler Method and RK-4 method reveals that both methods are convergent conditionally and may diverge for certain values of discretization parameter 'h', whereas the proposed NSFD scheme converges for all time step sizes and delay factors 'τ'. It also preserves all the essential properties of the continuous model.

7. CONCLUSION

During this study, it has been observed that an increase in the value of vaccination coverage level 'p', reduces the risk factor of spreading the disease by converting the susceptible population directly into the recovered population. Figure 6 to 9 show the effect of time delay on vaccinated model. It is observed that for same vaccination coverage levels, the increase in the value of τ can make the vaccination more effective by decreasing the number of infected individuals. As it is also realistic that if we vaccinate a population along with awareness and precautionary measures, there will be a rapid decrease in the infected population. Here the precautionary measures and awareness will act like a delay factor by providing more time to cure infected population form becoming infectious. Hence we can control any infectious disease more rapidly with the introduction of vaccination and awareness into a human population.

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Numerical Treatment of an Epidemic Model with Spatial Diffusion

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ABSTRACT

Structure preserving numerical methods are of great concern now a day. Many physical systems possess different properties which must be preserved by numerical method. For example, positivity is an important physical property possessed by different models. For instance, negative values for concentration of chemical reactions and subpopulations of an epidemic model cannot be negative. The purpose of this work is to propose a structure preserving numerical scheme for the solution of a reaction-diffusion epidemic model with specific nonlinear incidence rate. The proposed method is an implicit finite difference (FD) scheme. The proposed scheme is unconditionally dynamical consistent with positivity property. The proposed FD scheme is unconditionally convergent to the true steady states of the SIR reaction-diffusion system with specific nonlinear incidence rate. Comparison of proposed FD scheme is also done with the classical finite difference schemes to verify all the claims.

KEYWORDS: SIR epidemic model with specific nonlinear incidence rate; finite difference method; Convergence; Positivity.

1. INTRODUCTION

Mathematical epidemic models play a key role in the understanding of the dynamics of spread and control for the infectious diseases. Several mathematical epidemic models are presented in the literature to understand different infectious disease dynamics [3-7]. As nonlinearity on incidence rates of different diseases was examined, several writers have proposed the modification in standard bilinear incidence rate. In this work, we consider the following SIR reaction-diffusion epidemic model with specific nonlinear incidence rate for the numerical solution proposed by Al Mehdi Lotfi et al [2].

$$\left.\begin{aligned}\frac{\partial S}{\partial t} &= \lambda - \pi S - \frac{\beta SI}{1+\vartheta_1 S+\vartheta_2 I+\vartheta_3 SI} + \alpha_1 \frac{\partial^2 S}{\partial x^2}\\ \frac{\partial I}{\partial t} &= \frac{\beta SI}{1+\vartheta_1 S+\vartheta_2 I+\vartheta_3 SI} - (\pi+\delta+r)I + \alpha_2 \frac{\partial^2 I}{\partial x^2}\\ \frac{\partial R}{\partial t} &= rI - \pi R + \alpha_3 \frac{\partial^2 R}{\partial x^2}\end{aligned}\right\} \quad (1.1)$$

Where $S = S(x,t), I = I(x,t)$ and $R = R(x,t)$ are susceptible, infectious and recovered individuals respectively.λ is the recruitment rate, π is the natural death rate, δ is the death rate due to disease, r is the recovery rate of infected persons, β is infection parameter and $\beta SI/(1+\vartheta_1 S+\vartheta_2 I+\vartheta_3 SI)$ is the incidence rate, where $\vartheta_1, \vartheta_2, \vartheta_3 \geq 0$ are constants. Remember that the above incidence rate becomes bilinear if $\vartheta_i = 0, i = 1,2,3$ and the saturated incidence if $\vartheta_i = 0, i = 1,2$ or $i = 2,3$ [2].

In terms of numerical solutions for homogeneous and nonhomogeneous epidemic model, different authors proposed different numerical techniques [8-11]. But in this work our focus is to discuss a positivity preserving numerical techniques and propose an efficient positivity preserving numerical scheme for the solution of the reaction-diffusion epidemic system (1.1). Several authors presented different positivity preserving explicit and implicit numerical techniques for the ordinary and partial differential equations [13-17,19-24]. Mickens [12] presented rules to construct positivity persevering finite difference schemes, called nonstandard finite difference (NSFD) schemes. Mainly, he suggested that nonlinear term should replace with nonlocal approximation and discrete representation of derivative have non-trivial denominator functions. NSFD schemes are also applied on different epidemic models by several authors (for the readers, reference thereon [1,14-16,19]).

In this paper, a novel and efficient positivity preserving FD scheme is used to solve the reaction-diffusion epidemic system (1.1) which is proposed by Settapat Chinviriyasit and Wirawanchinviriyasit [17]. The proposed FD scheme is unconditionally stable, unconditionally positivity preserving and unconditionally convergent to

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the true steady states of the continuous system (1.1). Since R is not present in first two equations, so we can rewrite system (1.1) as

$$\frac{\partial S}{\partial t} = \lambda - \pi S - \frac{\beta SI}{1+\vartheta_1 S+\vartheta_2 I+\vartheta_3 SI} + \alpha_1 \frac{\partial^2 S}{\partial x^2}, \quad 0 < x < L \tag{1.2}$$

$$\frac{\partial I}{\partial t} = \frac{\beta SI}{1+\vartheta_1 S+\vartheta_2 I+\vartheta_3 SI} - (\pi + \delta + r)I + \alpha_2 \frac{\partial^2 I}{\partial x^2}, \quad 0 < x < L \tag{1.3}$$

With initial conditions

$$S(x,0) = \sigma_1(x) \text{ and } I(x,0) = \sigma_2(x) \tag{1.4}$$

And homogenous boundary conditions

$$S_x(0,t) = S_x(L,t) = 0, \tag{1.5}$$

$$I_x(0,t) = I_x(L,t) = 0, \tag{1.6}$$

2. Analysis of the model

The system (1.2) - (1.3) has two equilibrium points, disease free equilibrium (DFE) point and endemic equilibrium (EE) point [2]. The DFE point for the system (1.2) – (1.3) is $E_{DFE}(S_{DFE}, I_{DFE}) = E_{DFE}(\lambda/\pi, 0)$ and EE point is $E_{EE}(S_{EE}, I_{EE})$, where

$S_{EE} = \frac{2(e+\vartheta_2\lambda)}{\beta-\vartheta_1 e+\vartheta_2\pi-\vartheta_3\lambda+\sqrt{d}}$

And

$I_{EE} = \frac{\lambda-\pi S_{EE}}{e}$

With $e = (\pi + \delta + r)$ and $d = (\beta - \vartheta_1 e + \vartheta_2\pi - \vartheta_3\lambda)^2 + 4\vartheta_3\pi(e + \vartheta_2\lambda)$

Here, the reproductive number of the system [2] is

$$\Re_0 = \frac{\beta\lambda}{(\pi + \vartheta_1\lambda)(\pi + \delta + r)}$$

3. Numerical Modeling

In this section, three finite difference (FD) methods are used to solve the system (1.2) – (1.3), i.e backward Euler FD method, Crank Nicolson FD method and proposed positivity preserving FD method. We propose positivity preserving FD method for the numerical solution of the system (1.2) – (1.3) and then we will compare our results with a standard backward Euler and Crank Nicolson FD methods in this paper.

3.1. Backward Euler FD Method

Divide $[0, L] \times [0, T]$ into $M \times N$ with step sizes $h = \frac{L}{M}$ and $\tau = \frac{T}{N}$.

Grid points are

$x_i = ih, \quad i = 0,1,2,\dots,M,$

$t_n = n\tau, \quad n = 0,1,2,\dots,N,$

S_i^n and I_i^n are denoted as FD approximations of $S(ih, n\tau)$ and $I(ih, n\tau)$ respectively.

In the current section, we present backward Euler FD method for reaction-diffusion epidemic system (1.2) – (1.3) which is given as

$$(1 + 2\eta_1)S_i^{n+1} - \eta_1(S_{i-1}^{n+1} + S_{i+1}^{n+1}) = S_i^n + \lambda\tau - \tau\pi S_i^n - \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n+\vartheta_2 I_i^n+\vartheta_3 S_i^n I_i^n}$$

$$(1 + 2\eta_2)I_i^{n+1} - \eta_2(I_{i-1}^{n+1} + I_{i+1}^{n+1}) = I_i^n - \tau(\pi + \delta + r)I_i^n + \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n+\vartheta_2 I_i^n+\vartheta_3 S_i^n I_i^n}$$

3.2. Crank Nicolson FD Method

In this section, the Crank Nicolson FD scheme for the reaction-diffusion system (1.2) – (1.3) is given as

$$(1 + \eta_1)S_i^{n+1} - \frac{\eta_1}{2}(S_{i-1}^{n+1} + S_{i+1}^{n+1}) = (1 - \eta_1)S_i^n + \frac{\eta_1}{2}(S_{i-1}^n + S_{i+1}^n) + \lambda\tau - \tau\pi S_i^n - \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n+\vartheta_2 I_i^n+\vartheta_3 S_i^n I_i^n}$$

$$(1 + \eta_2)I_i^{n+1} - \frac{\eta_2}{2}(I_{i-1}^{n+1} + I_{i+1}^{n+1}) = (1 - \eta_2)I_i^n + \frac{\eta_2}{2}(I_{i-1}^n + I_{i+1}^n) - \tau(\pi + \delta + r)I_i^n + \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n+\vartheta_2 I_i^n+\vartheta_3 S_i^n I_i^n}$$

3.3. Proposed FD Method

Now we present proposed FD scheme for SIR reaction-diffusion epidemic system (1.2) – (1.3) with specific nonlinear incidence rate. The proposed FD scheme is implicit FD scheme which is given as

$$-\eta_1 S_{i-1}^{n+1} + (1 + 2\eta_1)S_i^{n+1} - \eta_1 S_{i+1}^{n+1} = S_i^n + \lambda\tau - \tau\pi S_i^{n+1} - \frac{\beta\tau S_i^{n+1} I_i^n}{1+\vartheta_1 S_i^n+\vartheta_2 I_i^n+\vartheta_3 S_i^n I_i^n} \tag{3.3.1}$$

$$-\eta_2 I_{i-1}^{n+1} + (1 + 2\eta_2)I_i^{n+1} - \eta_2 I_{i+1}^{n+1} = I_i^n - \tau(\pi + \delta + r)I_i^{n+1} + \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n+\vartheta_2 I_i^n+\vartheta_3 S_i^n I_i^n} \tag{3.3.2}$$

M-matrix theory [18] can be helpful to prove the positivity of the discretized system (3.3.1) – (3.3.2). A square matrix having real entries is call M-matrix if entries in off-diagonal are non-positive, entries in diagonal are

positive and matrix is strictly diagonally dominant. If a matrix is M-matrix, then it is singular. Inverse matrix of M-matrix always has the positive numbers entries.

Theorem 3.3.1 [19]
For any $h > 0$ and $\tau > 0$, the system (3.4.1) – (3.4.2) is positive, i.e. $S^n > 0$ and $I^n > 0$ for all $n = 0,1, \ldots$
Proof
The system (3.3.1) – (3.3.2) can be written as
$AS^{n+1} = B$and (3.3.3)
$CI^{n+1} = D$ (3.3.4)
Here A and B are square matrices of dimension $(N+1) \times (N+1)$. B and D are column matrices.

$$A = \begin{pmatrix} a_3 & a_1 & 0 & & 0 & 0 & 0 \\ a_2 & a_3 & a_2 & \cdots & 0 & 0 & 0 \\ 0 & a_2 & a_3 & & 0 & 0 & 0 \\ & \vdots & & \ddots & & \vdots & \\ 0 & 0 & 0 & & a_3 & a_2 & 0 \\ 0 & 0 & 0 & \cdots & a_2 & a_3 & a_2 \\ 0 & 0 & 0 & & 0 & a_1 & a_3 \end{pmatrix}$$

$$C = \begin{pmatrix} c_3 & c_1 & 0 & & 0 & 0 & 0 \\ c_2 & c_3 & c_2 & \cdots & 0 & 0 & 0 \\ 0 & c_2 & c_3 & & 0 & 0 & 0 \\ & \vdots & & \ddots & & \vdots & \\ 0 & 0 & 0 & & c_3 & c_2 & 0 \\ 0 & 0 & 0 & \cdots & c_2 & c_3 & c_2 \\ 0 & 0 & 0 & & 0 & c_1 & c_3 \end{pmatrix}$$

The off-diagonal entries and diagonal entries of A are $a_1 = -2\eta_1$, $a_2 = -\eta_1$ and $a_3 = 1 + 2\eta_1 + \tau\pi + \beta\tau I_i^n/(1 + \vartheta_1 S_i^n + \vartheta_2 I_i^n \vartheta_3 S_i^n I_i^n)$. The off-diagonal entries and diagonal entries of C are $c_1 = -2\eta_2$, $c_2 = -\eta_1$ and $c_3 = 1 + 2\eta_1 + \tau(\pi + \delta + r)$. Where $\eta_1 = \alpha_1\tau/h^2$ and $\eta_2 = \alpha_2\tau/h^2$. The entries of column matrix B are $S_i^n + \lambda\tau$ and entries of column matrix D are $I_i^n + \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n + \vartheta_2 I_i^n + \vartheta_3 S_i^n I_i^n}$. Also $S^n = (S_0^n, S_1^n, \ldots, S_M^n)^T$ and $I^n = (I_0^n, I_1^n, \ldots, I_M^n)^T$. Since $S_i^0 \geq 0$ and $I_i^0 \geq 0$ so $a_3 > 0$ and obviously $c_3 > 0$. Also $a_1, a_2, c_1, c_2 < 0$ and A, B are strictly diagonally dominant. From all the above conditions, it is concluded that A and C are M-matrices. This implies that A and C are non-singular matrices.So the equations (3.3.3) and (3.3.4) can be written as
$S^{n+1} = A^{-1}B$ (3.4.5)
$I^{n+1} = C^{-1}D$ (3.4.6)

Suppose that $S^n > 0$ and $I^n > 0$ and since A and C are M-matrix therefore all the entries of A^{-1} and C^{-1} are positive. So it is concluded that $S^{n+1} > 0$ and $I^{n+1} > 0$. Thus by induction, the system is positive.

3.4. Order and Consistency of Proposed FD Method

Accuracy of the proposed FD scheme can be obtained with the supposition of local truncation error as

$$\mathcal{L}_S[S, I: h, \tau] = \frac{1}{\tau}[S_i^{n+1} - S_i^n] - \lambda\tau + \tau\pi S_i^{n+1} + \frac{\beta\tau S_i^{n+1} I_i^n}{1+\vartheta_1 S_i^n + \vartheta_2 I_i^n + \vartheta_3 S_i^n I_i^n} - \frac{\alpha_1}{h^2}[S_{i-1}^n - 2S_i^n + S_{i+1}^n] - \left[\frac{\partial S}{\partial t} - \lambda + \pi S + \frac{\beta SI}{1+\vartheta_1 S + \vartheta_2 I + \vartheta_3 SI} - \alpha_1 \frac{\partial^2 S}{\partial x^2}\right] \quad (3.4.1)$$

$$\mathcal{L}_I[S, I: h, \tau] = \frac{1}{\tau}[I_i^{n+1} - I_i^n] + \tau(\pi + \delta + r) I_i^{n+1} - \frac{\beta\tau S_i^n I_i^n}{1+\vartheta_1 S_i^n + \vartheta_2 I_i^n + \vartheta_3 S_i^n I_i^n} - \frac{\alpha_2}{h^2}[I_{i-1}^n - 2I_i^n + I_{i+1}^n] - \left[\frac{\partial I}{\partial t} - \frac{\beta SI}{1+\vartheta_1 S + \vartheta_2 I + \vartheta_3 SI} + (\pi + \delta + r) I - \alpha_2 \frac{\partial^2 I}{\partial x^2}\right] \quad (3.4.2)$$

The Taylor's series expansion of S_i^{n+1},S_{i-1}^{n+1},S_{i+1}^{n+1},I_i^{n+1},I_{i-1}^{n+1} and I_{i+1}^{n+1} are

$$S_i^{n+1} = S_i^n + \tau\frac{\partial S_i^n}{\partial t} + \frac{\tau^2}{2!}\frac{\partial^2 S_i^n}{\partial t^2} + \frac{\tau^3}{2!}\frac{\partial^3 S_i^n}{\partial t^3} + \cdots,$$

$$S_{i+1}^{n+1} = S_i^n + \tau\frac{\partial S_i^n}{\partial t} + h\frac{\partial S_i^n}{\partial x} + \frac{\tau^2}{2!}\frac{\partial^2 S_i^n}{\partial t^2} + \frac{h^2}{2!}\frac{\partial^2 S_i^n}{\partial x^2} + \tau h\frac{\partial^2 S_i^n}{\partial x \partial t} + \cdots,$$

$$S_{i-1}^{n+1} = S_i^n + \tau\frac{\partial S_i^n}{\partial t} - h\frac{\partial S_i^n}{\partial x} + \frac{\tau^2}{2!}\frac{\partial^2 S_i^n}{\partial t^2} + \frac{h^2}{2!}\frac{\partial^2 S_i^n}{\partial x^2} - \tau h\frac{\partial^2 S_i^n}{\partial x \partial t} + \cdots,$$

$$I_i^{n+1} = I_i^n + \tau\frac{\partial I_i^n}{\partial t} + \frac{\tau^2}{2!}\frac{\partial^2 I_i^n}{\partial t^2} + \frac{\tau^3}{2!}\frac{\partial^3 I_i^n}{\partial t^3} + \cdots,$$

$$I_{i+1}^{n+1} = I_i^n + \tau\frac{\partial I_i^n}{\partial t} + h\frac{\partial I_i^n}{\partial x} + \frac{\tau^2}{2!}\frac{\partial^2 I_i^n}{\partial t^2} + \frac{h^2}{2!}\frac{\partial^2 I_i^n}{\partial x^2} + \tau h\frac{\partial^2 I_i^n}{\partial x \partial t} + \cdots,$$

$I_{i-1}^{n+1} = I_i^n + \tau\frac{\partial I_i^n}{\partial t} - h\frac{\partial I_i^n}{\partial x} + \frac{\tau^2}{2!}\frac{\partial^2 I_i^n}{\partial t^2} + \frac{h^2}{2!}\frac{\partial^2 I_i^n}{\partial x^2} - \tau h\frac{\partial^2 I_i^n}{\partial x \partial t} + \cdots,$

Substituting the values of S_i^{n+1},S_{i-1}^{n+1},S_{i+1}^{n+1},I_i^{n+1},I_{i-1}^{n+1} and I_{i+1}^{n+1} in (3.4.1) and (3.4.2) and after simplifications we have,

$$\mathcal{L}_S[S, I: h, \tau] = -\frac{1}{12}\alpha_1 h^2\frac{\partial^4 S}{\partial x^4} + \tau\left[\frac{1}{2}\frac{\partial^2 S}{\partial t^2} + \pi\frac{\partial S}{\partial t} + \frac{\beta I}{1+\vartheta_1 S+\vartheta_2 I+\vartheta_3 SI}\frac{\partial S}{\partial t} - \alpha_1\frac{\partial^3 S}{\partial x^2 \partial t}\right] + \cdots, \quad (3.4.3)$$

$$\mathcal{L}_I[S, I: h, \tau] = -\frac{1}{12}\alpha_2 h^2\frac{\partial^4 I}{\partial x^4} + \tau\left[\frac{1}{2}\frac{\partial^2 S}{\partial t^2} + (\pi+\delta+r)\frac{\partial S}{\partial t} - \alpha_2\frac{\partial^3 S}{\partial x^2 \partial t}\right] + \cdots \quad (3.4.4)$$

From equations (3.4.3) and (3.4.4), it is verified that proposed FD method is $O(h^2+\tau)$ as $h, \tau \to 0$.

3.6. Stability of Proposed FD Method

To prove that the proposed scheme is unconditionally stable, we use Von Neumann stability method. For this purpose, we substitute $\zeta(t+\Delta t)e^{i\propto x}$, $\zeta(t)e^{i\propto x}$, $\zeta(t+\Delta t)e^{i\propto(x-\Delta x)}$ and $\zeta(t+\Delta t)e^{i\propto(x+\Delta x)}$in S_i^{n+1},S_i^n,S_{i-1}^{n+1} andS_{i+1}^{n+1} in equation (3.3.1). After linearizing and simplifications, we have

$$\left|\frac{\zeta(t+\Delta t)}{\zeta(t)}\right| = \left|\frac{1}{1+4\eta_1\sin^2(\propto\Delta x/2)+\tau\pi+\tau\beta}\right| < 1 \quad (3.6.1)$$

In similar way, for equation (3.3.2)

$$\left|\frac{\zeta(t+\Delta t)}{\zeta(t)}\right| = \left|\frac{1+\tau\beta}{1+4\eta_2\sin^2(\propto\Delta x/2)+\tau(\pi+\delta+r)}\right| < 1 \quad (3.6.2)$$

From equations (3.6.1) and (3.6.2), it is proved that proposed scheme is unconditionally stable.
In similar way, it can be proved that Crank Nicolson FD scheme and backward Euler FD scheme are unconditionally stable.

Table 1 (Disease Free Equilibrium$R_0 < 1$)

Parameter	λ	π	δ	β	r	ϑ_1	q_2	ϑ_1	ϑ_2	ϑ_3	α_1	α_2
Value	0.5	0.1	0.1	0.2	0.5	0.1	0.2	0.1	0.02	0.03	0.1	0.5

Table 2 (Endemic Equilibrium$R_0 > 1$)

Parameter	λ	π	δ	β	r	ϑ_1	q_2	ϑ_1	ϑ_2	ϑ_3	α_1	α_2
Value	0.5	0.1	0.1	0.6	0.5	0.1	0.2	0.1	0.02	0.03	0.1	0.5

4. Numerical experiment:

Now by using the values of parameters given in the table 1 and table 2 [2]we execute numerical experiment for all finite difference schemes. For this purpose, we take system (1.1) – (1.2) with homogeneous boundary conditions and initial conditions [2]

$$S(x,0) = \begin{cases} 1.1x, & 0 \le x < 0.5 \\ 1.1(1-x), & 0.5 \le x < 1 \end{cases}$$

$$I(x,0) = \begin{cases} 0.5x, & 0 \le x < 0.5 \\ 0.5(1-x), & 0.5 \le x < 1 \end{cases}$$

4.1. Backward Euler FD Scheme:

First we present the graphs of backward Euler FD scheme for both disease free equilibrium and endemic equilibrium.

4.1.1. Disease Free Equilibrium:

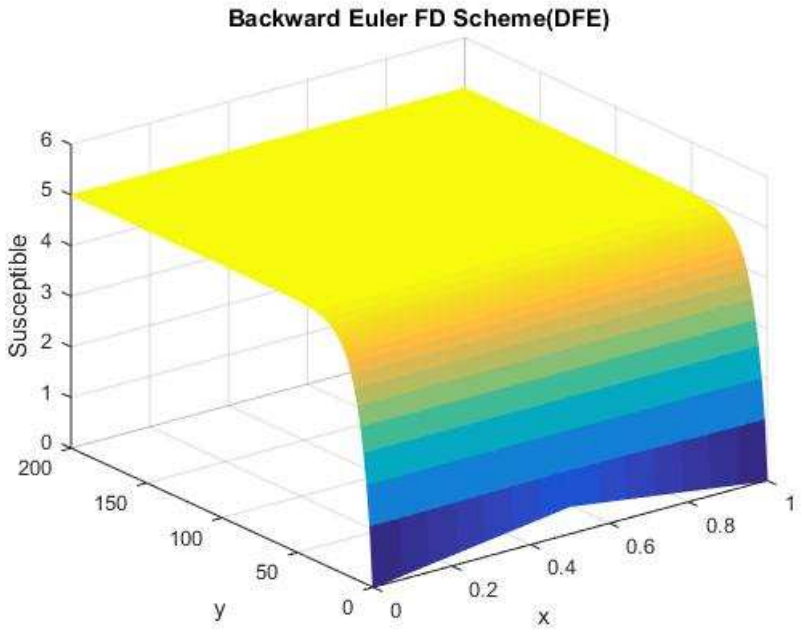

Figure 1(a)

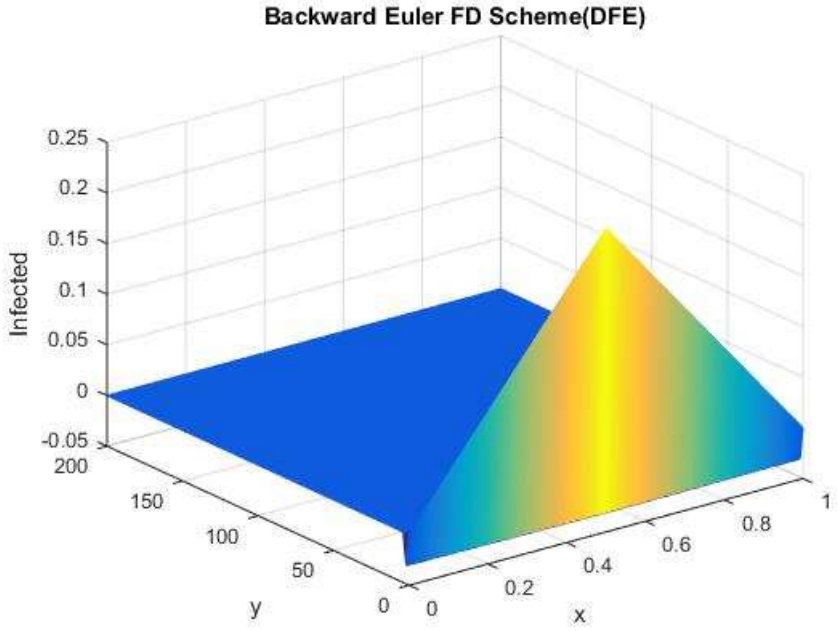

Figure 1(b)

Figure 1: **Figures 1(a) – 1(b) represent the graphs of susceptible and infected individuals for disease free equilibrium using backward Euler FD scheme at $h = 0.001$, $\eta_1 = 2 \times 10^{-5}$ and $\eta_2 = 10^{-6}$.**

Figure 1 shows that backward Euler FD scheme produces negative values of infected individuals which is meaningless in population dynamics. Therefore, backward Euler FD scheme fails to preserve positivity property possessed by system (1.2) – (1.3).

4.1.2. Endemic Equilibrium:

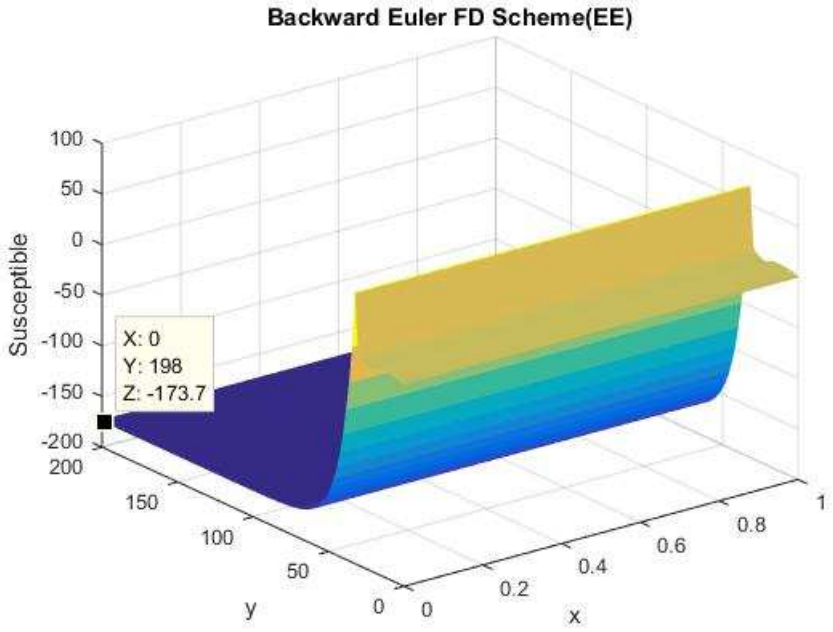

Figure 2(a)

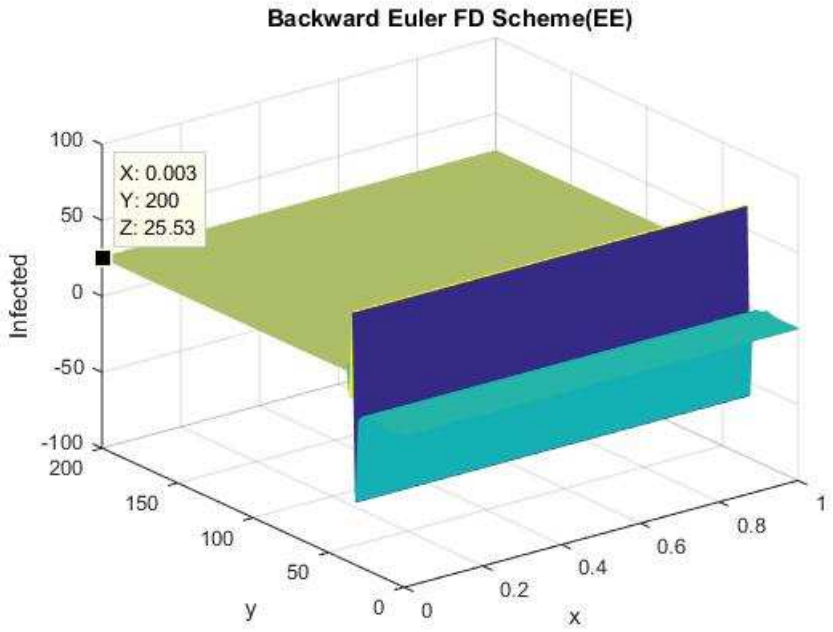

Figure 2(b)

Figure 2: Figures 2(a) – 2(b) show the graphs of susceptible and infected individuals for endemic equilibrium using backward Euler FD scheme at $h = 0.001$, $\eta_1 = 2 \times 10^{-5}$ and $\eta_2 = 10^{-6}$.

Figure 2 magnifies the graphs for endemic equilibrium of susceptible and infected individuals using backward Euler FD scheme. Figures 2(a) and 2(b) show that backward Euler FD scheme not only produces negative values of susceptible and infected individuals but also converges to false equilibrium point. Note that endemic equilibrium point is $E_{EE}(S_{EE}, I_{EE})$. After substituting the values of parameters in endemic point, we get $E_{EE}(S_{EE}, I_{EE}) = E_{EE}(1.3625, 0.5196)$. It can be verified from the figure 2 that backward Euler FD scheme converges to false endemic equilibrium point.

4.2. Crank Nicolson FD Scheme:

Now we present the graphs of Crank Nicolson FD scheme for both disease free equilibrium and endemic equilibrium.

4.2.1. Disease Free Equilibrium:

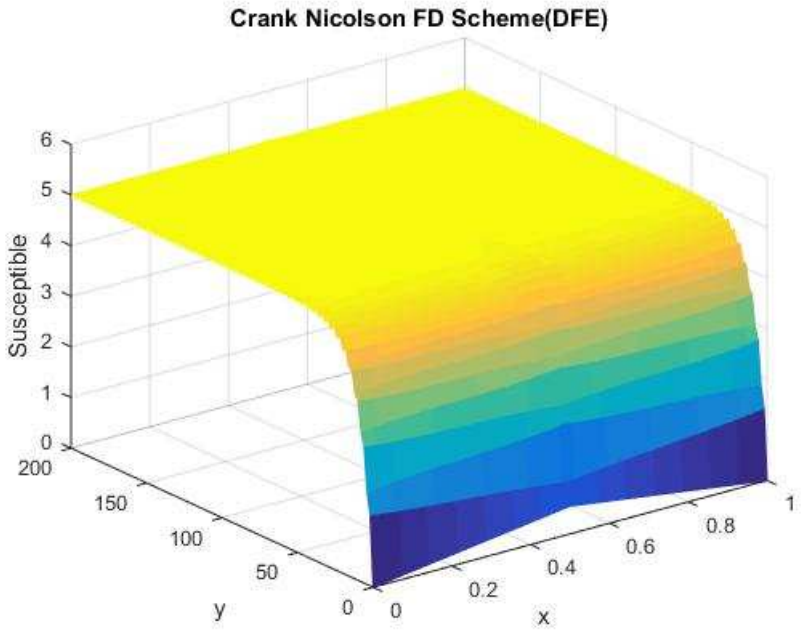

Figure 3(a)

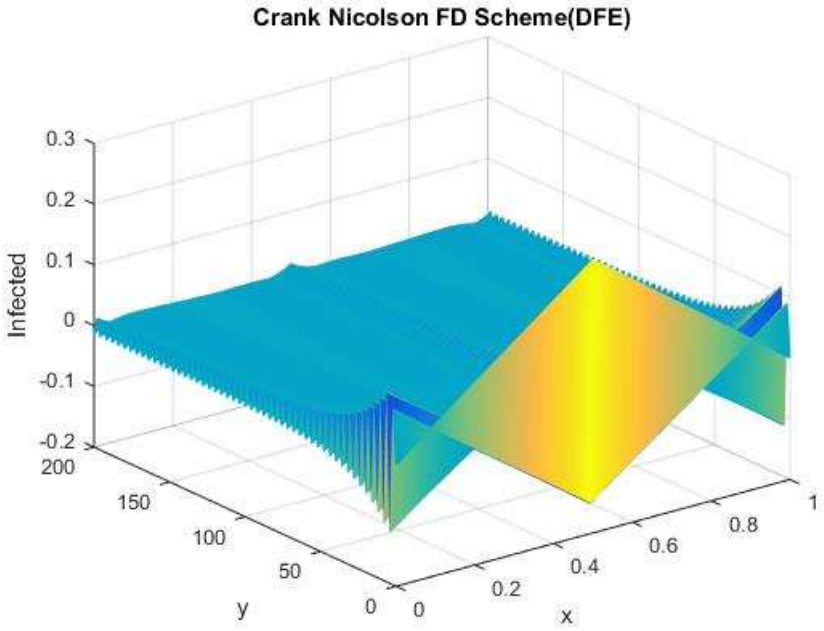

Figure 3(b)

Figure 3: Figures 3(a) – 3(b) represent the graphs of susceptible and infected individuals for disease free equilibrium using Crank Nicolson FD scheme at $h = 0.001$, $\eta_1 = 2 \times 10^{-5}$ and $\eta_2 = 10^{-6}$.

Crank Nicolson FD scheme also loses the positivity property as shown in the graph of infected individuals in figure 3(b).

4.2.2. Endemic Equilibrium:

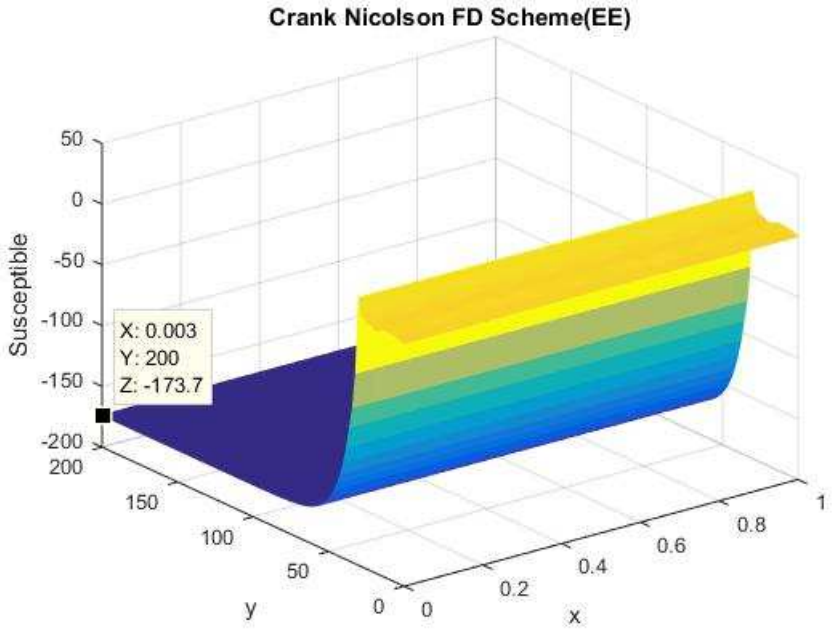

Figure 4(a)

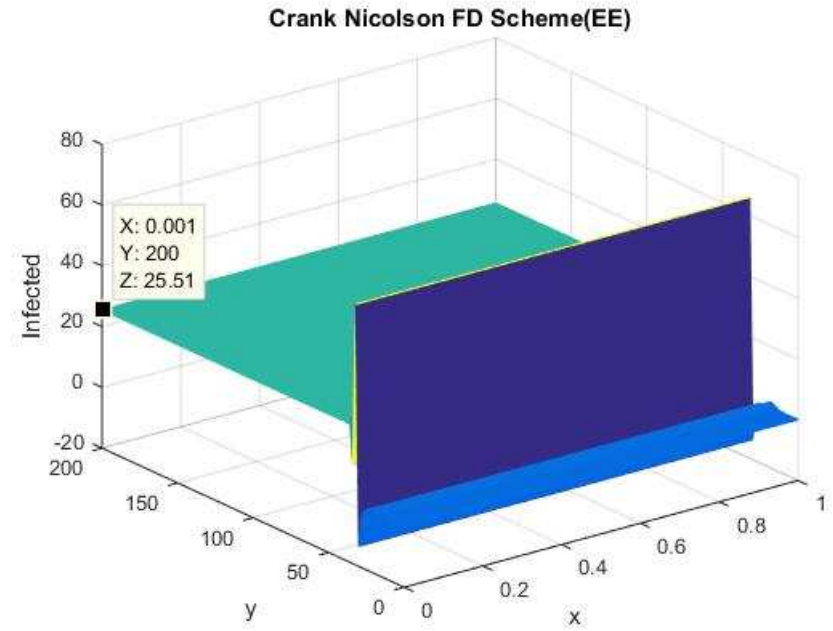

Figure 4(b)

Figure 4: Figures 4(a) – 4(b) represent the graphs of susceptible and infected individuals for endemic equilibrium using Crank Nicolson FD scheme at $h = 0.001$, $\eta_1 = 2 \times 10^{-5}$ and $\eta_2 = 10^{-6}$.

The graphs of susceptible and infected individuals in figure 4(a) and 4(b) indicate the failure of structure preserving properties by Crank Nicolson FD scheme, as Crank Nicolson FD scheme shows the negative behavior and converges to false equilibrium point.

4.3. Proposed FD Scheme:

Now we discuss the behavior of proposed FD scheme and present the simulations. First we present the graphs of initial distributions for susceptible and infected individuals.

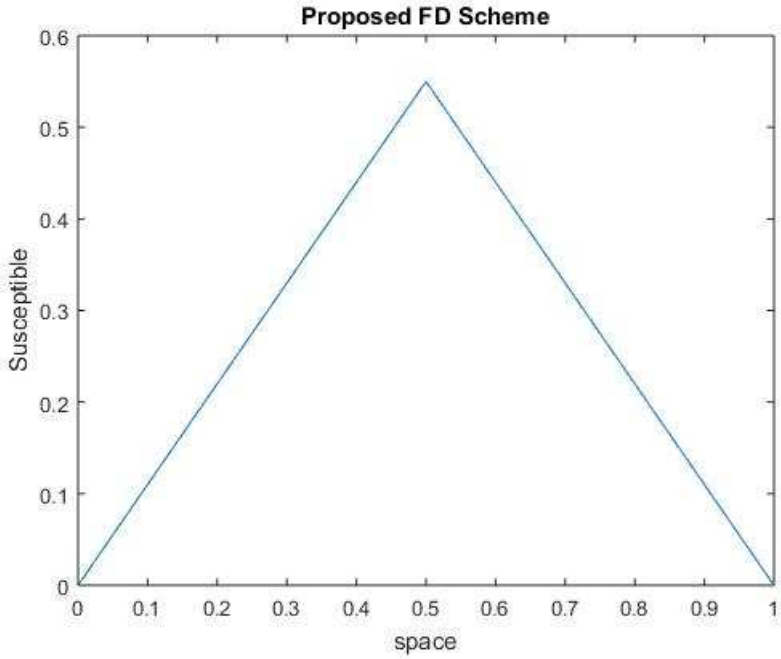

Figure 5(a)

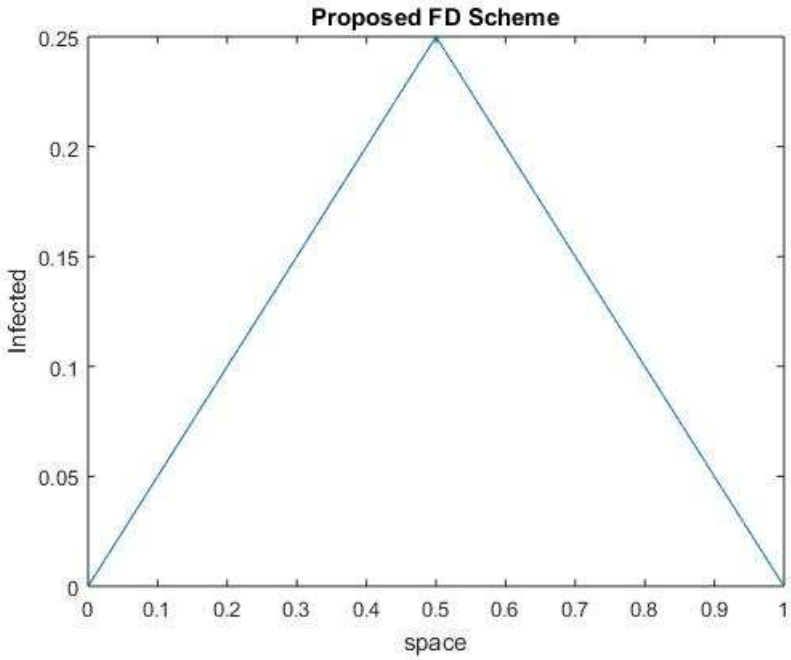

Figure 5(b)

Figure 5:Figures 5(a) – 5(b) reveals the graphs of susceptible and infected individuals for initial distributions.

Figure 5 reflects that the concentration of susceptible and infected individuals is maximum at the middle of the interval [0,1].

4.3.1. Disease Free Equilibrium:

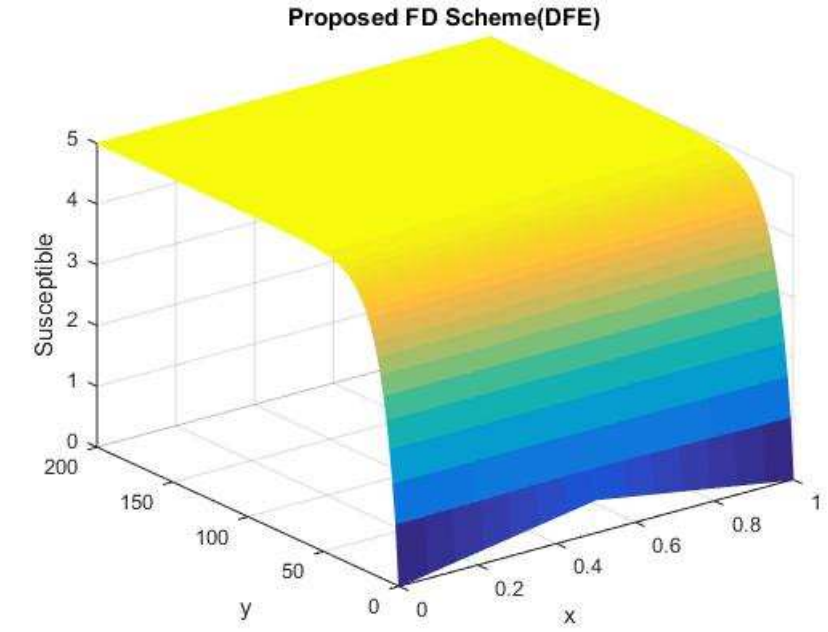

Figure 6(a)

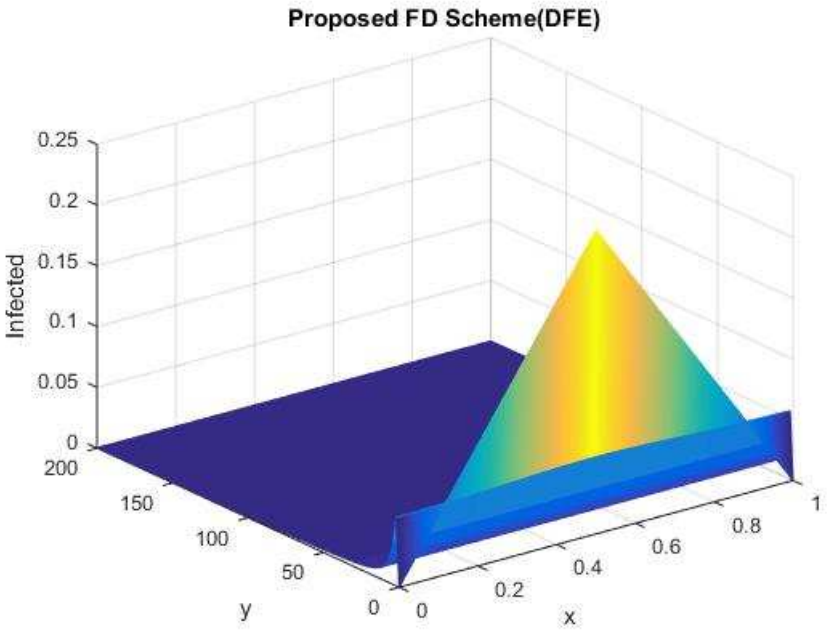

Figure 6(b)

Figure 6:Figures 6(a) – 6(b) represent the graphs of susceptible and infected individuals for disease free equilibrium using proposed FD scheme at $h = 0.001$, $\eta_1 = 2 \times 10^{-5}$ and $\eta_2 = 10^{-6}$

Figure 6 verifies the statement of theorem 3.3.1 as proposed FD scheme shows the positive behavior for both susceptible and infected individuals. Also proposed FD scheme converges to the disease free equilibrium point $E_{DFE}(S_{DFE}, I_{DFE}) = E_{DFE}(\lambda/\pi, 0) = E_{DFE}(5,0)$.

4.3.2. Endemic Equilibrium:

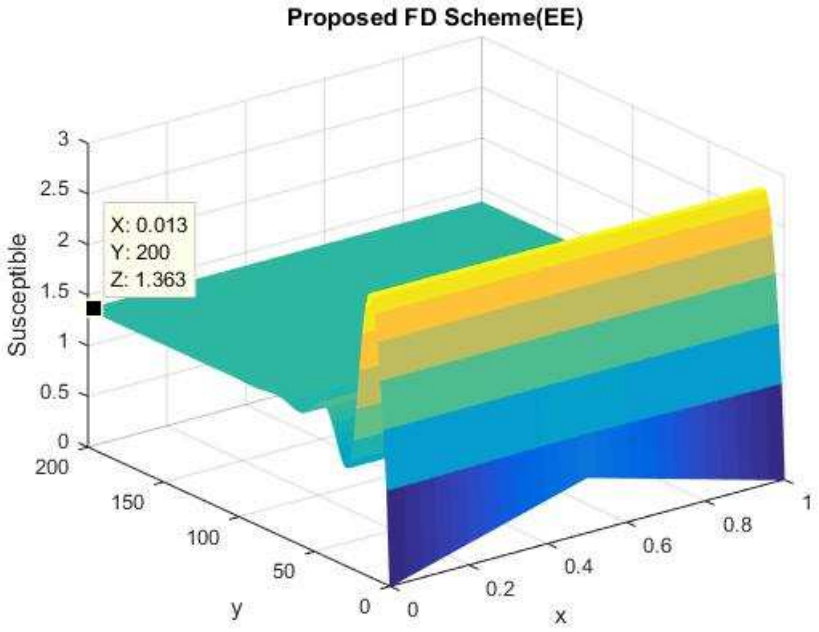

Figure 7(a)

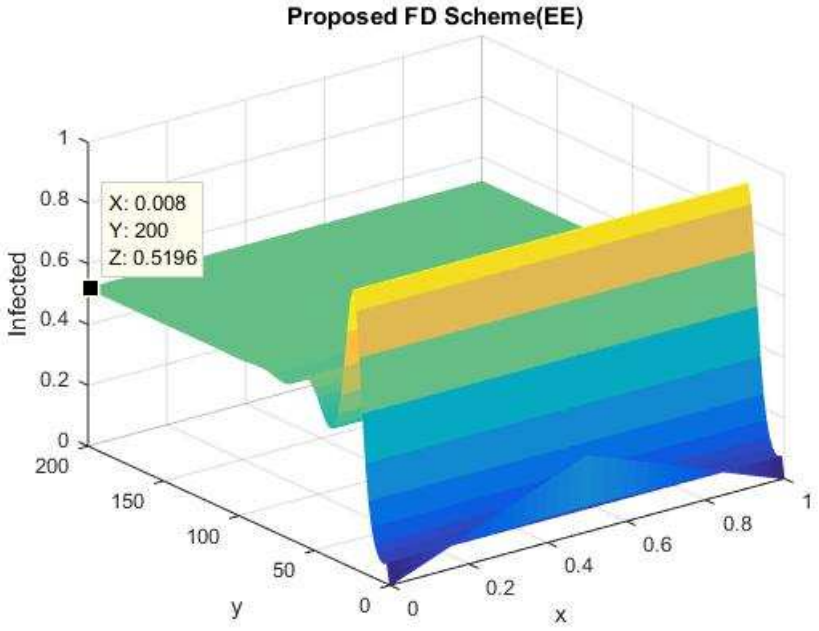

Figure 7(b)

Figure 7:Figures 7(a) – 7(b) represent the graphs of susceptible and infected individuals for disease free equilibrium using proposed FD scheme at $h = 0.001$, $\eta_1 = 2 \times 10^{-5}$ and $\eta_2 = 10^{-6}$.

The verification of the theorem 3.3.1 is shown in the figure 7 as the proposed FD scheme preserves positivity property. Also proposed scheme converges to the endemic equilibrium point $E_{EE}(S_{EE}, I_{EE}) = E_{EE}(1.3625, 0.5196)$ as shown in figure 7(a) and 7(b).

5. CONCLUSION

This paper is concerned about the numerical solution of reaction-diffusion epidemic model with specific nonlinear incidence rate. In this article, we developed a structural preserving implicit finite difference scheme

for SIR reaction-diffusion epidemic model with specific nonlinear incidence rate. The proposed FD scheme is unconditionally dynamically consistent with positivity property. Also proposed FD scheme unconditionally converges to the true steady states (equilibrium points) of the continuous model. We also presented the convergence analysis of the proposed FD scheme and proved that proposed NSFD scheme is unconditionally stable and consistent with the help of Von Neumann stability analysis and Tayler series expansion respectively. We also proved in theorem 3.1.1 by induction that proposed FD scheme preserves positivity. The results are compared with the well-known backward Euler implicit FD scheme and Crank Nicolson implicit FD scheme. Both classical FD schemes fail to preserve positivity property, give non-physical behavior and converge to false steady states. On the other side proposed NSFD scheme is unconditionally convergent to true steady states. Simulations are done to verify all the claims of the proposed FD scheme.

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In Vitro Maize Growth Promotion by Endophytic *Fusarium Oxysporum* WLW

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ABSTRACT

Fungal endophytes are well-unknown for their potential to improve plant growth and health by production of bioactive compounds including indole-3-acetic acid (IAA) and ammonia. In current study we isolated an endophytic fungus wlw from leaves of *Watheniasomnifera* growing under xeric conditions. The isolate was identified as strain of *Fusarium oxysporum* by sequencing internal transcribed spacer regions (ITS) of 18S rDNA and phylogenetic analysis. Screening of culture filtrate (CF) revealed that the strain was capable to produce IAA and ammonia in considerable amount. Application of CF of the strain significantly enhanced maize growth demonstrated by higher shoot and root length and fresh and dry plant biomass as compared to the control plants supplied with endophyte free medium or water. In conclusion, *F.oxysporum* wlw has significant potential of plant growth promotion and hence recommended for field trials as potential candidate for use as bio fertilizer to improve plant growth and yield in sustainable agriculture.
KEY WORDS: endophytic fungi, plant growth promotion, *Fusariumoxysporum* IAA, ammonia

INTRODUCTION

Endophytic fungi are found asymptomatically in plant tissues in virtually all plant species [1]. The interaction between endophyte and host is mutualistic or neutral and possibly on different hosts and according to environmental conditions [2]. Endophytes are microorganisms (fungi or bacteria) that are widespread in plant tissues, inhabiting their host asymptomatically. A symbiotically relationship is maintained between host and endophyte throughout the life cycle of the host, beginning with germination of the seed and persist till seed set[3]. These fungal symbionts can profoundly affect plant ecology, adaptability and evolution [4], and are important for community structure and associated biodiversity [5]. Endophytic fungi provide a number of benefits to the host plants in various ways, such as promotion of growth, protection against diseases and pests, and augmenting absorption of minerals**.** Host colonization by endophytic fungi enhances the ecological adaptability of the plant by improving its tolerance against the biotic and abiotic stresses [1]. Such benificial endophytes are the members of a large family of organisms known as plant growth promoting microorganisms (PGPMs). Ability of these endophytes to mobilize insoluble soil phosphorus, improve nitrogen availability and produce phytohormones including IAA make them excellent candidates to be used as bio-fertilizers [6]. Plants and microorganisms, including bacteria, algae and fungi, can yield IAA [7]. The role of microbial IAA in plant-microbe communications has recently received increasing attention. In addition, several studies have shown that IAA is a signaling molecule in microorganisms affecting gene expression in numerous microorganisms [8]. They can affect vital physiological features of plant protecting it against biotic and abiotic stresses[9,10]. Under extreme environmental conditions, endophytes may secrete biologically active compounds such as plant hormones that can bring greater benefits to host plants [9], while on the other hand they are environment friendly and can be introduced for commercial application. Fungi may play an important role in plant survival by enhancing nutrient uptake and producing growth promoting metabolites such as gibberellins and auxins. *Penicillium* and *Trichoderma* strains are known to produce a number of beneficial compounds to inhibit pathogens [11] and stimulate plant growth by producing plant hormones [12] and/or degradation of complex substrates [13]. Likewise, *Penicillium* and *Aspergillus* have been reported to produce gibberellins, which are growth regulators in higher plants [12,14]. The current study was aim to isolate endophytic fungi from leaves of *Watheniasomnifera* and to screen the isolates for production of plant growth promoting substances (IAA and ammonia) and their potential for *in-vito* plant growth promotion.

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MATERIALS AND METHODS

Plant material and isolation of endophytic fungi

The healthy leaves of *Watheniasomnifera* were collected from drought stress area of district Mardan. The plant materials were brought to laboratory in sterile polythene bags and were processed within 24 hours to diminish the risks of contamination. The collected leaf materials were rinsed with tap water and were then surface sterilized with 5% sodium hypochlorite solution for 5 seconds followed by 95% ethanol for 3 min and finally rinsed 5 times with autoclave double distill water and were air dried under sterile condition to remove excess moisture. After surface sterilization, the root materials were cut approximately into 0.5 cm pieces using a flame sterilized scalpel. About 5 to 6 segments were placed on Hegam medium plates (0.5% glucose, 0.05% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.05% NH_4Cl, 0.1% $FeCl_3$, 80ppm streptomycin and 1.5% agar; pH 5.6±0.2) for one week (Hamayun et al., 2010). For purification the developing fungal plugs were grown on potato dextrose agar (PDA) medium plates. For production of culture filtrate and biomass, the purified fungus was then grown in 250 mL flask containing czapekbroth medium (50 ml; 1% glucose, 1% peptone, 0.05% KCl, 0.05% MgSO4.7H2O, and 0.001% FeSO4.7H2O; pH 7.3±0.2) for seven days at 28 °C and 120 rpm in shaking incubator [15]. Colonization frequency (CF %) of the isolated strain was determined as described previously [16,17]. Colonization frequency was equal to number of segments colonized by endophyte/total no of segments observed*100.

Screening of isolates for plant growth promoting characters including ammonia and IAA

The isolated strains were screened for the production of ammonia as described earlier [18]. Endophytes were grown in 15 mL czapek broth medium contained in test tubes, under previously described conditions. After 7 days, CF was obtained as mentioned above and 0.5 mL of Nessler's reagent was added after to it. Appearance of brown color indicated the presence of ammonia in the CF. For IAA production salkowski reagent tests was adopted [19].

DNA extraction and molecular characterization of the isolated strain

Fresh mycelium was collected and fungal genomic DNA was extracted using the SolGent Fungus Genomic DNA Extraction Kit (Cat No. SGD64-S120; SolGent Co., Daejeon, Korea) as described previously [20]. The primers NS1 5' (GTA GTC ATA TGC TTG TCT C) 3' and NS2 5' (AAA CCT TGT TAC GAC TTT TA) 3' were used for the PCR. In a 30 µL reaction mixture, PCR was performed using 20 ng of genomic DNA as a template using EF-Taq (SolGent, Korea) as follows: Taq polymerase was activated at 95°C for 2 minutes and 35 cycles of 95°C for 1 minute each, 55°C and 72°C for 1 minute, completing the 10-minute step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).

Plant growth promotion assay

Fungal inocula were prepared by growing the endophytes in Czapek broth under previously described conditions. The culture was harvested after seven days by centrifugation to separate the pure CF and mycelium. Maize seeds were dipped in water for 30 min and then surface sterilized with 0.1% mercuric chloride solution and finally rinsed 5 times with sterilized distilled water. Filter paper was cut according to the shape of petri plates. Two-fold filter paper was placed in petri plates and autoclaved. To avoid contamination, surface sterilized seeds were placed in autoclaved petri plates with the help of sterilized forceps and were allowed to germinate. After germination, the seedlings were transferred to autoclaved petri plates as described earlier [21]. Five plants per treatment were grown for two weeks. The freeze-dried CF was diluted with 1 mL autoclaved distilled water. Each plant received 10 µL of CF at two leaf stage.For comparison, two kinds of control plants received (i) of endophyte-free medium and (ii) distilled water. After two weeks of growth different growth characteristics such as shoot length, root length and fresh and dry weight was determined.

Statistical analysis

Software SPSS for windows 16.0 (SPSS Inc., Chicago, IL, USA) was used to compare means by one-way analysis of variance (ANOVA) and Duncan multiple range test ($p = 0.05$).

RESULTS AND DISCUSSSION

Isolation and preliminary screening of endophytic fungi

A total of 3 endophytes were isolated from the leaves of the selected plants. two plants were used to obtain 15 leaves segments yielding 3 endophytes after an incubation period of 7 days. Dominant strain was wlw1, sprouted out from 9 root segments showing highest colonization frequency (65%) of the isolated strains (Table 1). The strains wlw, wlw2 were least common showing (15%) colonization frequency. Only the wlw strain wasfound positive for production of both IAA ammonia. The production of IAA, ammonia suggested that wlw may be suitable candidates for plant growth promotion and was therefore selected for further study. Fungal endophytes yield bioactive metabolites that promote the plant endophyte interaction [22]. Promoting plant growth is the most important effect of fungal symbiosis [23], where endophytic fungi promote plant growth by producing various secondary metabolites, including ammonia and plant hormones, particularly IAA [24].

Molecular identification of the selected strain

To identify the strain, its DNA was extracted for subsequent amplification and sequencing ITS region. Sequence of the ITS region near the 18 S rDNA was obtained and was subjected to homology analysis using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The obtained sequence showed 99% homology and 100% query cover with that of *Fusariumoxysporum*. Identification of the strain was confirmed by carrying out phylogenetic analysis of the sequences of closely resembling endophytes retrieved from NCBI GenBank, using MEGA 7.0 software. The neighbor-joining tree based upon ITS sequence homology grouped our strain with *Fusariumoxysporum* (Figure 1). Sequence was submitted to GenBank under the accession No MH005071.

Plant growth promotion assay

Maize seedlings grown in Petri plates for one week were exposed to 10µL of filter sterilized fungal culture filtrate (obtained by growing endophytes in Czapek broth for 7 days). For comparison, maize seedlings were treated with same volume ofendophytes free czapek medium and dH_2O [14]. Maize seedlings in all the sets were allowed to grow for 7days and growth parameters were then recorded. All the growth parameters including shoot and root length, fresh and dry weight were significantly enhanced by endophyte wlw in comparison to endophytes free czapek medium and water applied controls (Figure 2a and b). The strain wlw improved shoot growth by two fold in comparison to endophyte free medium control. Previously [25] reported thatendophytic*F. oxysporum* increase plant height 11.3% which was lower than increase in shoot length caused by our isolate.Also root growth was further enhanced by the endophyte and maize seedlings exposed to their culture filtrate had roots which 26% longer than the media control respectively. Similarly, Czapek media showed stimulatory effect on the production of fresh biomass by maize seedlings. The stimulatory effect of Czapek medium on maize fresh biomass was further enhanced by the secondary metabolites of endophyte. For example, CF of wlw caused maize seedlings to produce 31%, more fresh biomass than the media control. BlankCzapekmediumalsoenhancedmaizedryweightthanthewatercontrol (Figure 2b). Indole acetic acid as an essential compound for the growth and development of shoot and roots, many microorganism including plant growth promoting rizobacteria (PGPR) produce IAA [26]. Similarly seedling dry biomass was further enhanced by endophyte wlw by 62% in comparison to endophytes free czapek medium.Previously [27] also observed the positive effect of endophyte on banana growth, they reported that endophytic fungi *Fusarium oxysporum* increased height of plant, pseudostem diameter and number of leaves. Enhanced growth through endophytes may be the result of phytohormones produced by fungal endophytes such as in maize [28].

Table 1. Characterization of endophytic fungi isolated from *W. somnifera* for plant growth promoting characters including IAA and ammonia production along with their colonization frequency.

Plant	Strains	IAA	Ammonia	Colonization %
Watheniasomnifera	Wlw1	+	-	60
Watheniasomnifera	Wlw	+	+	15
Watheniasomnifera	Wlw2	+	-	15

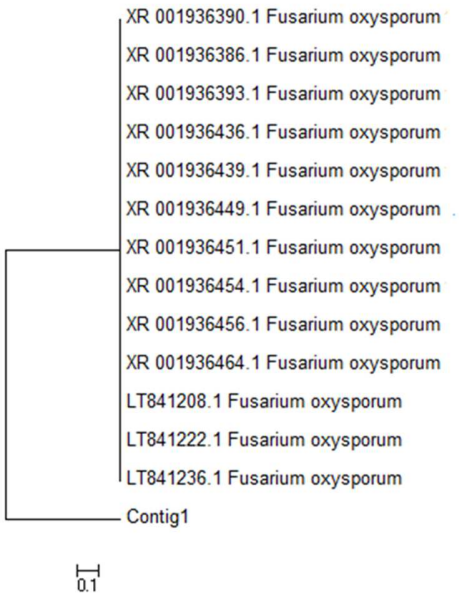

Figure 1. Phylogenetic tree constructed with neighbor joining method with 18S rDNA sequence (ITS region) of *Fusariumoxysporum*. Fungal isolate wlw formed a sub clade with *Fusariumoxysporum*.

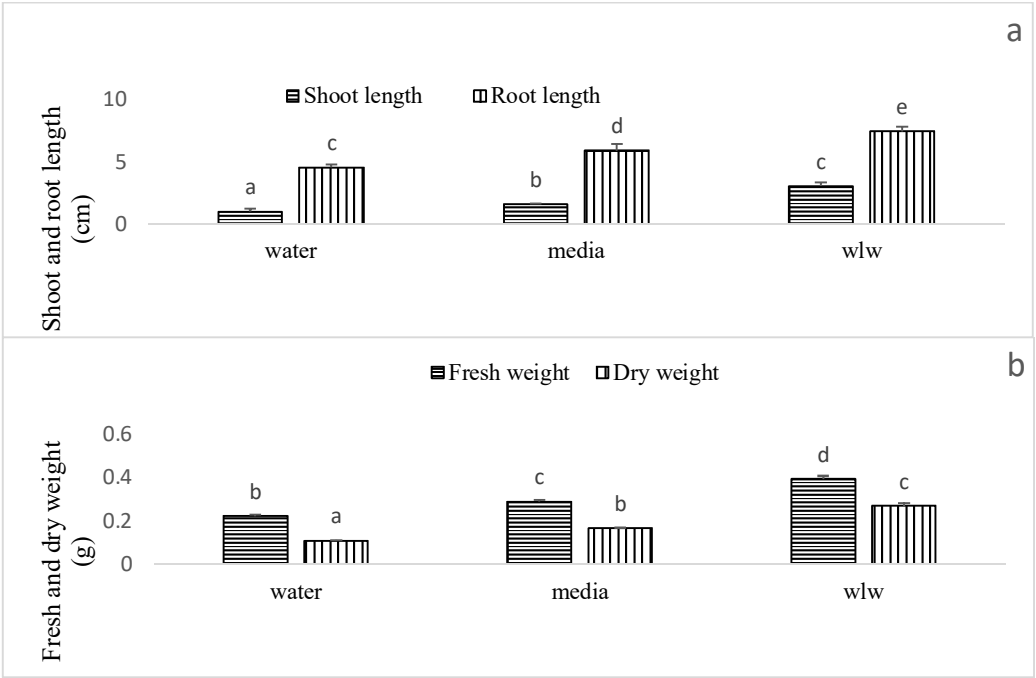

Figure 2. Effect of endophyte culture filtrate on the a) shoot length and root length (b), fresh biomass and dry biomass of maize seedlings grown in petri plates for 2 weeks. Data are mean of 9 replicates from 3 independent experiments with standard error bars. Bars labelled with different letters are significantly different (Duncan test; $p<0.05$).

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Characteristics of Particle Board Composite of Natural Fiber from *Musa Acuminate L.* That Was Increased in Abstract Position with Resin Polymer Matrix

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ABSTRACT

This research started with the removal of banana bark fiber and then did the pretreatment that is process to eliminate lignin in raw material. It used NaOH with variation 0%; 3%; 5% and 7%, after that printing with abstract composition. The characteristic test performed on this particle board is physical characteristic with parameters used are density, moisture content, water absorption every 24 hours and 72 hours and the development of thickness at immersion 24 hours and 72 hours. The density values obtained from each composition are 0.7696 g/cm^3; 0.7776 g/cm^3; 0.7824 g/cm^3; 0.7872 g/cm^3. The resulting water content is 15.1839%; 7,1073%; 6.2544%; 7,0472%. Absorption of water obtained in abstract position with 0% NaOH concentration; 3%; 5% and 7%, on the immersion of 24 hours generated 20.7125%; 11.7536%; 10,3788; 11.5477%. Meanwhile, on immersion 72 hours generated 24.7819%; 11.9799%; 10,8888%; 11.9789%. The value of thick development obtained at 24 hours of immersion that is 98.2465%; 43,7819%; 31.7889%; 36,1121%, while the thick development value obtained at 72 hours of immersion that is 98,4819%; 43.8129%; 32.1588%; 37.9569%.
KEYWORDS: Banana fiber, Particle Board Composite Materials, Pretreatment, Physical Characteristics.

INTRODUCTION

Increasing population causes the need for building/construction[1] and for household furniture[2] to increase, even faster than the population growth itself. The need for solid wood as industrial and construction[1] raw materials is increasing as the population grows. The shortage of supply of solid wood needs to be anticipated because it will endanger the sustainability of the forest on one side and the continuity of the industry on the other hand One way to overcome this by substituting solid wood with non-timber materials that are still not optimally utilized. One of them is waste of banana stem fiber (Musa Acuminate L.). The availability of such materials in Indonesia is quite abundant, so the opportunity of utilization as raw material of composite material[1,2,3,4,5,6,7] is very possible. If this technology can be developed it will increase the use value of banana stem waste, so that banana stem waste is not just a waste from plantation but can be utilized to be something more economical[3].

Composites are materials formed by the combination of two or more different components[12]. In general, composites are composed of two material[1,2,3,4,5,8,9,10,11] components, namely material matrix and reinforcement or amplifier, the two parts of this material are interconnected with each other based on the function of each part. The substrate or filler serves to strengthen the matrix because in general the substrate is much stronger than the matrix and will reinforce the formation of the material by affecting the physical and mechanical properties of the material formed. While the polymer matrix serves as a substrate protector rather than environmental effects and collision damage[13]. In addition, the composite material[1,2,3,4,9,10,11] comprises 2 or more phases (matrices phase and dispersed phase) and has properties significantly different from each component. Matrices phase is the primary phase which has continuous character. Matrices are usually more elastic and slightly hard. The matrix holds the dispersed phase and divides its load by the dispersed phase. The dispersed phase (reinforcement) is embedded within the matrix in a non-continuous form. The dispersed phase is usually stronger than the matrix, so it is sometimes called the booster phase[14].

Preparation of this composite[2,4,7,8,13,14] material beforehand must go through a treatment that is the process of delignification is done for fiber uptake because if the banana stalk is used without treatment / delignification will cause the surface of the fibers to become dirty and formed wax that can cause the surface of the fiber becomes softer, so the fiber bond with the matrix become weak and decrease the tensile strength[20], one of the fluids that can be used for the treatment process is NaOH.

Natural Fiber

Fiber is a strong material[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19], stiff, brittle. Because the fibers mainly resist the outer forces, there are two things that make the fiber resist the force that is: bonding between the fiber and the matrix (intervarsial bonding) is very good and strong so it is not easily separated from the matrix (debonding), aspec ratio the ratio between fiber length and fiber diameter is quite large. Fiber is characterized by its very high modulus and strength, elongation (good span

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strength), good heat stability, the ability to be transformed into filaments and a number of other properties that depend on usage[29].

In general it can be said that the function of fiber is as a reinforcement material to strengthen the composite [2,5,15,20,24,25,26,28,29] so that its mechanical properties are more rigid, tough and stronger than with no reinforcing fibers, in addition to fiber also save the use of resin. In the combination of fibers and resins, the fibers act as reinforcements which typically have high strength and stiffness, while the resin serves as an adhesive or matrix to maintain the position of the fibers, transmits shear forces and also serves as a fiber coating[30].

According Chandrabakty[31] there are several reasons to use natural fiber[2,23]as a composite amplifier as follows:

a. More environmentally friendly and biodegradable compared to synthetic fibers
b. Natural fiber weight is smaller
c. It has a weight-modulus ratio better than E-glass fiber
d. Natural fiber composites have higher acoustic damping power than E-glass fiber composites and carbon fibers
e. Natural fibers are more economical than glass fibers and carbon fibers

The requirement for fiber placement and different fiber directions makes fiber-reinforced composites differentiated into several parts [14] :

a. Continuous fiber composite (composite reinforced with fibers continue)
   Continuous or uni-directional, has a long and straight fiber arrangement, forming a lamina between the matrices. This type of composite is most commonly used. This type has a weakness in the separation between layers. This is because the power between layers is influenced by the matrix
b. Woven fiber composite (composite reinforced with fiber webbing)
   This composite is not easily influenced by the separation between layers because the fiber arrangement also binds between layers. However, the composition of the longitudinal fibers that are not so straight result in strength and stiffness will weaken. The composite consists of a matrix layer followed by a layer of fiber webbing
c. *Discontinuous Fiber Composite*
   Composites with short, random-type fibers are often used in large volume production because of their cheaper manufacturing cost factors. The disadvantages of this type of random fiber are the mechanical properties that are still below the strengthening with the straight fibers on the same fiber type.
d. *Hybrid fiber composite*
   Hybrid fiber composite is a composite combination of straight fiber types with random fibers. This type is used in order to replace the deficiencies of the two types and can combine the advantages.

Banana Fiber Composition(Musa Acuminate L.)

Banana fiber obtained from the banana tree kepok (Musa Acuminate L.) is a fiber that has good mechanical properties. The mechanical properties of banana stem fiber have a density of 1.35 g / cm 3, the cellulose is 63-64%, hemicellulose (20%), 5% lignin content, 600 Mpa average tensile strength, average tensile modulus 17,85 Gpa and the increase of length 3,36%[32].

Pretreatment

The purpose of pretreatment is to open the lignocellulosic structure so that there will be structural changes both physically and chemically. In this study, the pretreatment process uses an alkali solution ieNaOH. According to Sreekala et al.[33], the alkaline solution will provide high stability and maximum moisture retention.

Polymer Resin Matrix

The properties of the polymer will determine the exact application. The main advantages of the polymer as a matrix are low price, easy processing, good chemical resistance and low density. Conversely, low strength, low modulus and low operating temperatures limit their use[34]. Polymer composites are very popular because of their low price and simple manufacturing methods. Strengthening the polymer with a strong fiber network, has the following characteristics[22]:

a. High strength
b. High stiffness
c. High resistance to breaking / breaking
d. Good abrasion resistance
e. Good impact resistance
f. Good corrosion resistance
g. Resilience to fatigue (fatigue) is good
h. Low cost

While the major disadvantages of composite polymers are:

a. Low thermal resistance
b. High thermal expansion coefficient

Commonly used polymers are thermoplastic polymers, thermosetting polymers, elastomers and mixtures thereof, but in this study polymer resins are included in the thermosetting polymer classes. In the liquid thermoset resin polymers are converted into hard and brittle solids formed by chemical crosslinks forming very strong polymer chains. Thermoset resins do not melt due

to heating. At the time of printing, this resin does not need to be applied pressure, because when it is liquid it has a relatively low viscosity, hardened at room temperature with the use of a catalyst without producing gas (unlike other thermoset resins)[15].

The mechanical properties of the composite[2,5,15,20,24,25,26,28,29] particle board can be known by examining the physical properties of the composite particle board. Physical testing can be:

1. **Density**
   To find out the physical properties of composite particle board, density test (ρ) was done. The mass density of a homogeneous material is defined as the mass of volume unity. Mathematically writable:
   $$\rho = \frac{m}{v}$$
   Where :
   ρ = density (gr/cm^3);
   m = mass of the test sample (gram);
   v = volume of test sample (cm^3)

2. **Water content**
   Particle board moisture content calculated from the initial weight and final weight after a drying in an oven for 24 hours.composite particle board moisture content calculated using the formula:
   $$\text{Water content} = \frac{BA-BB}{BB} x\ 100\%$$
   Where :
   KA = moisture content (%)
   BA = initial weight of test sample (gram)
   BB = fixed weight of test sample after drying (gram)

3. **Water Absorption**
   At the moment there is the possibility to form a sample of air trapped in the layer of aggregate or occur due to decomposition of minerals that formed due to weather changes, then formed small holes or cavities in the aggregate grains (pore). The pores in the sample vary and spread throughout the granules. The pores may be a free water reservoir inside the aggregate. The percentage of water weight absorbed by aggregates and fiber in water is called water absorption[26], while the amount of water contained in aggregate and fiber is called water content[35].
   $$\text{Water Absorption} = \frac{Mb-Mk}{Mk} x100\%$$
   Where :
   Mb = initial mass
   Mk = final mass

4. **Thick Development**
   The thick development[40] is defined as a quantity that expresses the thickness of the test sample in percent against the initial thickness. The thick development is measured using the formula:
   $$\text{Thick Development} = \frac{T2-T1}{T1} x100\%$$
   Where :
   T1 = Thick Initial
   T2 = Thick end

MATERIAL AND METHOD

Raw Material Preparation

Raw material such as banana bark is cleaned and dried for 1 week, then crushed with a wire brush and drawn fiber. After the fiber was obtained, the fiber was immersed in NaOH solution with each concentration of 3%, 5% and 7% for 2 hours. Then the fiber is washed with aquadest and drained.

Composite Manufacture

The banana fiber is arranged in abstract mold, then doused with a resin matrix according to the predefined composition.

Composite Testing

Tests carried out in this study were density[27]values, water content, water absorption[26] and thick development[40] using Japanese Industrial Standard (JIS) standards for Particleboard A 5905-2003[36] and Japanese Industrial Standard (JIS) for Particleboard A 5908-2003[37].

RESULTS AND DISCUSSION

Composite Particle Density

One of the physical properties of composite[3,5,15,16,21,22,23,24,25,26,27] boards that greatly affect the mechanical properties of the composite particle board is density[27]. The result of measurement of density value on composite particle board in this study ranged between 0,7696 g/cm^3 – 0,7872 g/cm^3.

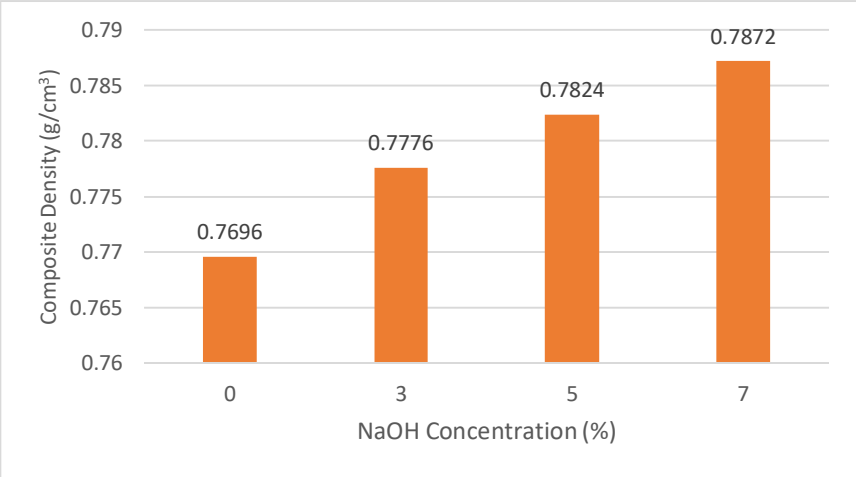

Figure 1. Graph of the relationship between the density values of the composite particle board with concentration of NaOH

Based on Figure 1, it can be seen that the density test results on composite board particles produced the highest density of 0.7872 g/cm^3 at 7% NaOH concentration and the lowest density 0.7696 g/cm^3 at 0% concentration. This suggests that composite boards fall into categories meeting the standards required by JIS A 5908-2003[37] which require a density of between 0.4 g/cm^3-0.9 g/cm^3. As well as the category the density required to meet the standards JIS A 5905-2003[36] which require density fiberboard ranging from 0.35 g/cm^3- 0.8 g/cm^3. In the composite particle board that has been through a treatment process using NaOH showed a greater density than the absence of prior treatment. According to JIS A 5908-2003 [37] for composite particle board is included in the medium density category.

Water content of Composite Particle Board

One of the physical properties that shows the water content of the composite particle board in a state of equilibrium with its environment is the moisture content.

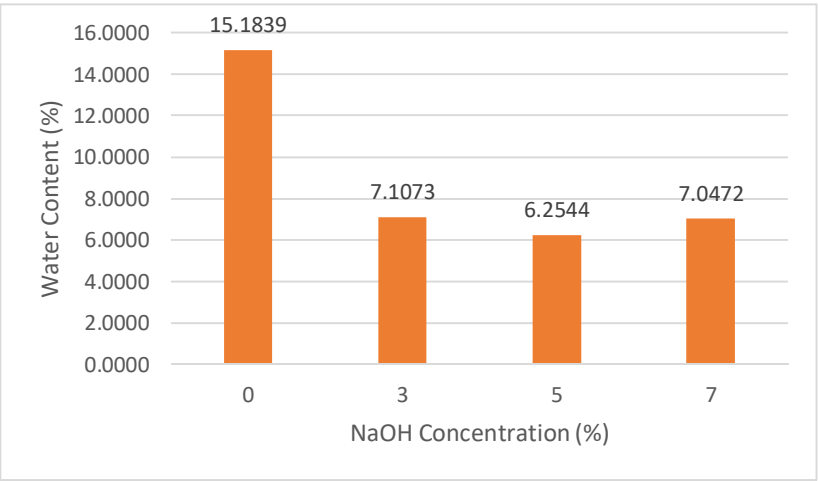

Figure 2. Graph of the relationship between the water content of the composite particle board with the concentration of NaOH

Based on Figure 2, the highest water content of 15.1839% was obtained at 0% NaOH concentration (without NaOH treatment) and the lowest water content was 6.2544% with 5% NaOH concentration. This indicates that the composite particle board meets the requirements of JIS A 5908-1994[37] and JIS A 5905-1994[36] for the fiberboard which requires the particle board and board values between 5% -13%.

According to Haygreen and Bowyer[38] that the water content of raw materials greatly determines the water content of the resulting composite board, the higher the water content of raw materials, the higher the water content of composite boards because not all water vapor can be removed from the composite board. In the manufacture[3,5,6,11,19] of composite board raw materials must be dry with water content of about 2% -5%, so that if added adhesive then the water content of raw materials will increase up to 4% -6%. Composite board without treatment shows high water content, this is because banana bark fiber has hygroscopic properties, but also due to drying of raw materials only about 2 hours. This is done to avoid damage to the fiber is relatively small. Meanwhile, banana stem fiber treated with NaOH reacts to form a highly reactive alkaline cellulose that, according to Kollmann and Cote[39], the composite board will have high water repellent properties. Hence it can cause hygroscopic properties on the composite particle board to decrease.

Water Absorption

One of the physical properties of composite[3,5,15,16,21,22,23,24,25,26,27] particle board that shows the ability to absorb water is the water absorption.In this study the immersion was done at 24 hours and 72 hours.

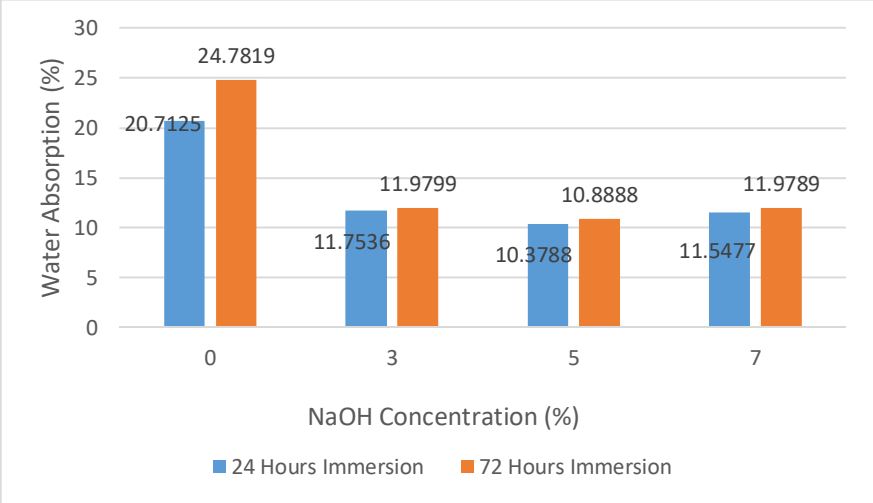

Figure 3. Graph of the relationship between water absorption capacity of composite particle board with NaOH concentration

Based on Fig. 3, the highest water absorption value was 20.7125% at 24 hours and 24.7819% for immersion for 72 hours with 0% NaOH concentration (without NaOH treatment) and lowest absorption of 10.3788% at soaking for 24 hours and 10,8888% at immersion for 72 hours with 5% NaOH concentration. This may be due to the banana midstring fiber having hygroscopic properties. However, in banana stem fibers treated with NaOH will react to form highly reactive alkaline cellulose so that according to Kollmann and Cote[34] the composite board will have high water repellent properties. Hence it can cause hygroscopic properties on the composite particle board to decrease. However, JIS A 5908-1994[37] and JIS A 5905-1994[36] do not require water absorption on composite boards.

Thick Development

The nature of this thick development[40] is usually used as a reference to determine whether a composite board is used as an interior or exterior type composite board.

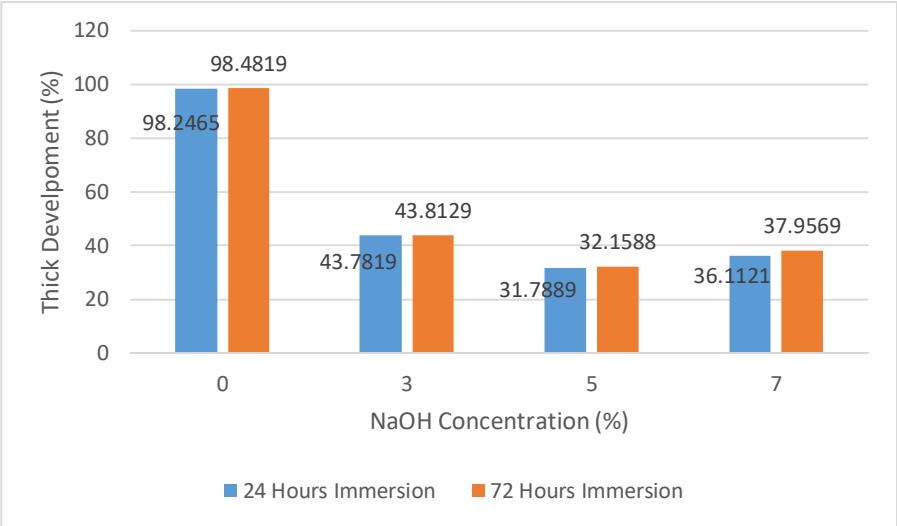

Figure 3. Graph of the relationship between thickness development of composite particle board with NaOH concentration

Based on Fig. 4, the highest thick development value of 98.2465% was achieved on immersion for 24 hours and 98.4819% for immersion for 72 hours with 0% NaOH concentration (without NaOH treatment) and lowest absorption of 31,7889% at immersion for 24 hours and 32.1588%.

JIS A 5908-1994 standard requires a maximum of 12% thick development while in JIS A 5905-1994 does not require the development of thick. In this study all the samples showed less qualified results in JIS A 5905-1994. This is thought to be because the psori midrib is very hygroscopic and includes a type of plant that has high water content. However, treatment using NAOH may have an effect on lowering the thickness rate even if the results are below standard.

CONCLUSION

Based on the results of the research, the following conclusions are obtained:

1. The density of the composite particle board produced the highest density of 0.7872 g/cm^3 at 7% NaOH concentration and the lowest density 0.7696 g/cm^3 at 0% concentration. This composite particle board belongs to the category compliant with JIS A 5908-1994 which requires a density of between 0.4 g/cm^3- 0.9 g/cm^3 for particleboard and also meets the required density standard JIS A 5905-1994 which requires a board the density fibers range from 0.35 g/cm^3 to 0.8 g/cm^3 so that the composite particle board is of medium density category.
2. The highest moisture value was 15,1839% at NaOH concentration 0% (without NaOH treatment) and lowest water content (best) equal to 6,2544% with 5% NaOH concentration. The composite particle board with NaOH treatment meets the requirements of JIS A 5908-1994 and JIS A 5908-1994 which requires a particle board particle value between 5% -13%. However, non-NaOH composite particle board does not meet the requirements required by JIS A 5908-1994 and JIS A 5905-1994.
3. The highest water absorption value was 20.7125% at immersion for 24 hours and 24.7819% for immersion for 72 hours with 0% NaOH concentration (without NaOH treatment) and the lowest absorbent (best) of 10.3788% at immersion during 24 hours and 10,8888% at immersion for 72 hours with 5% NaOH concentration. JIS A 5908-1994 and JIS A 5905-1994 do not require water absorption on composite boards.
4. Highest development value of 98.2465% for immersion for 24 hours and 98.4819% for immersion for 72 hours with 0% NaOH concentration (without NaOH treatment) and lowest absorbance (best) of 31.7889% at immersion for 24 hours and 32.1588%. Overall this composite particle board does not meet the requirements of JIS A 5908-1994 which requires the development of a maximum thickness of 12. Whereas, for the JIS A 5905-1994 fiber composite board does not require the development of thick.

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Effect of Parental Conflicts on Adolescents Personality Development in Pakistan

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ABSTRACT

There is no relationship entirely free from conflict and disagreement. Parental conflict is harmful to adolescents. When parents are involved in conflicts, children demonstrate sorrow. Their responses are terror, anxiety, annoyance, sadness, depression as well as they are at high threat of bad personality development. The main objective was to investigate the parental conflict and its bad impact on adolescent personality development within demographic and cultural context. A research was led in urban ranges in the District of Faisalabad, Punjab, Pakistan. A sample of 400 respondents (adolescents age 10-19 years, having both parents) was taken through multistage sampling technique. A well-made questionnaire was prepared to investigate the research goals. Data analysis, which is based upon univariate and bivariate (chi-square and gamma text) was used to evaluate the responses and its association with the bad effect on personality development. The result of quantitative study indicated that the majority proportion of those respondents who had a high extent of parental conflict in their homes, had high levels of bad effect on their personality development. The value of chi-square is significant at (0.000). Among the sample, age, family type, sibling size, reason of parental conflict, extent of parental conflict, drug addiction and health problems were strongly related to the bad outcome of adolescent personality development. It is recommended that parents should realize that their adolescents are hurting by experiencing the parental conflict. There should be a psychologist or counselor to secure children's personality from the bad effect of parental conflicts.

KEYWORDS: Interparental harmony, Parent-adolescent relationship, Demographic factors, Psychological effects, character development, emotional security, cultural conditions.

INTRODUCTION

Parents perform an immense responsibility of personality development of their children. They build a solid base for their offspring consecutively to have a flourishing and rewarding life. The foundation needs to be built at a premature age, as well as requirement to continue being built all the way through the child's early adolescence. There are diverse ways of socializing adolescents and of bringing up children. These behaviors, foster positive or negative thoughts, therefore promoting societal development. There is a connection between poor child rearing (disregard/dismissal) with low levels of compassion. The absence of compassion is emphatically identified with the harsh parental conduct [1]. A classic study demonstrated that authoritative parenting involving high warmth, responsiveness and communication, led to the most positive emotional, social and rational expansion in adolescents [2]. There is sure ecological setting that a mother can give to improve the advancement furthermore the fitness of their adolescents. It's a basic piece of human baby to wind up plainly appended to a mother figure, in light of the fact that solid mother-adolescent connection is a noteworthy precursor of the early enthusiasm for others and can be a fundamental precondition for the personality improvement. The mother-adolescent connection assumes a focal part in the advancement of professional social conduct. The expression of fathering shows up with regards to the more straightforward mental and physical part, a man institutes in the raising of his youngsters.

The development of the personality is the representation of the child of the societal world in addition to the self and emotional plus social fitness [3]. Parents who are familiar with how to modify the child by nurturing strategy to manage the exacting behavior of their adolescent can greatest give direction along with assuring the fruitful progression of their child's character. Character is similarly subject to a man's moral advancement [4]. Adolescents gain knowledge of behaviors, principles, rules as well as the actions of the members of their family. The warm relationships of parents and families nurture their child's learning and development. The base of the adolescents' knowledge is upon parent-child interaction. With parents responsive along with conventional support, offspring's build up the qualities they require to be successful in life. The premature association between parents and children have influential effects on children's emotional well-being [5]. Parents who are

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appraised high on warmth demonstrate the more elevated amount of concern, association and love in the direction of their adolescents [6].

A single variable that most accurately predicts youngster wrongdoing is the youthful person relation with his/her guardians. The better the relation is, the less likely the adolescent will become delinquent [7]. Family, the most important unit of society, when does not function adequately, the individual feels anxious, frustrated, dissatisfied, insecure or even hostile towards family members. The home condition fundamentally influences the adolescent's psychological well-being [8]. A satisfying marital relationship is the cornerstone of good family functioning. The affectionate parent-child relationships have been found when spouses are mutually supportive. On the other hand, a home environment that is characterized by quarreling, nagging and disagreement has a very bad effect on both parents and children.

Adolescents don't like parental conflicts, but when situation alters against their wish, it shows negative effects on current and future life of the children. Parental hostility towards youngsters is emphatically connected with inter-spousal animosity [9]. The primary motivation behind the investigation is to recognize the demographic along cultural factors and its association with the development of the adolescent's personality in the urban areas of Pakistan.

Theoretical Framework

There are a number of factors which affect personality development of the adolescent. The theoretical framework explains factors that affect personality development. In the present study, Sameroff's transactional model [10] as well as Davies and Cummings theory of social security [11] are applied to understand and support the relationship between the variables. Here, the theoretical model of personality development is built up on the basis of these theories.

Figure 1: Theoretical Model of Personality Development

Conceptual Framework

The study covered seven demographic and cultural variables to examine the relationship connecting personality development and parental conflict. Here, the model of conceptual framework gives reference points to talk of text, method in addition to analysis as well as the data interpretation.

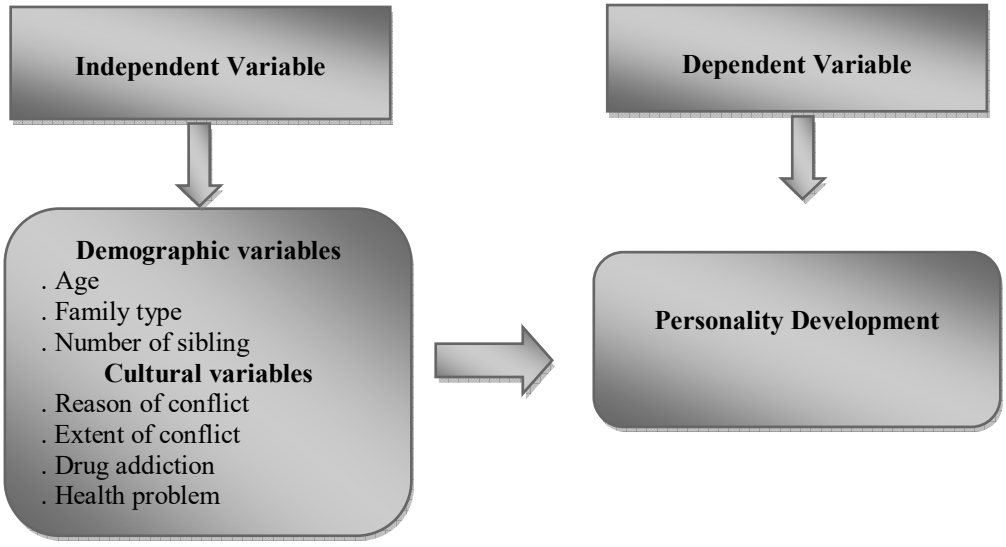

Figure 2: Conceptual Model of Personality Development

Objectives of the study

1. To identify the demographic and cultural factors of personality development among respondents
2. To find out the effect of parental conflicts on personality development of respondents
3. To see the relationship between parental conflicts and personality development

Hypothesis of the study

1. An association between higher age and bad effect on personality development
   (Older children may furthermore be more delicate to conflict)
2. An association between family type and bad effect on personality development
   (Adolescents belong to nuclear family have good personality development than joint family)
3. An association between the higher number of sibling and bad effect on personality development
   (Having many children is exhausting)
4. An association between reasons of inter-parental conflict and awful effect on the development of the personality
   (The dangerous clash between guardians has durable, negative consequences for adolescents)
5. A connection between level of inter-parental conflict and bad outcome in the development of the personality
   (Adolescents' contact with harsh, however peaceful, the clash between guardians additionally applies negative impacts on child improvement)
6. A connection linking drug addiction and bad effect on personality development
   (Adolescents start self injurious activities to curb the stress and anxiety)
7. An association between health problem and bad effect on personality development
   (Conflicted home environment creates unhealthy adolescents)

LITERATURE REVIEW

Literature review develops the extent of the inquiry. It shows how a research statement towards previously available work. The literature of review of the most significant variables is presented at this point to enhance the value of the research work. Albert et al. [12] found that children are more vulnerable to parental conflict in the age of adolescence. This period of development shows major threat to children's health, development, and safety. Bilal et al. [13] evaluated the relationship between family structure and personality development. The most important factor for personality development is the home environment. The responsibility of parents is extra significant than any other affiliate of the relation in nuclear and joint family system. Caceres-Delpiano [14] also concluded that family size affects child wellbeing. In the context of Pakistani culture, Those houses where resources are limited, child well being is restricted. A large number of siblings in a family have a pessimistic impact on personality development. Big family size increases the likelihood of parental conflict.

According to Ochsner [15] the most common conflict is because of work and family. Amato and Perviti [16] found that parental conflict leads to divorce and there are many reasons behind it. These reasons varied by gender, social class and life course variables. In Pakistan, a spouse blames the other than own self for the problem that led to divorce. The problem with in-laws, extramarital sex, conflict over children and lack of communication are most highlighted reasons of conflict. Nazir et al. [17] expressed that parents are role models for their children and children be likely to copy the behavior of their parents in their own associations. Audrain-McGovern et al. [18] concluded that parental conflict leads adolescents towards depression. Depression is the leading factor of drug addiction as well as mental and physical illness.

METHODOLOGY

This research empirically investigated how the parental conflict affecting adolescent personality development. The quantitative approach was used to measure the effect of parental conflict on adolescent's personality development. The District Faisalabad is selected as a study area. The District of Faisalabad was randomly chosen through simple random sampling technique. A research was organized in Faisalabad's four urban towns. The area of the research was comprised of numerous union councils of urban towns of the District Faisalabad, which were randomly selected. The purpose of selected urban towns was to represent the utmost difference in data. A sample of 400 respondents (adolescents) was selected through the technique of multistage sampling. At the first stage, four towns were conveniently selected. Secondly, four union council from each town were chosen randomly. Thirdly, 25 respondents (adolescents) age 10-19 years and having both parents were selected randomly from each selected union council to investigate the objectives of the research. All information was collected by using a questionnaire. The SPSS (Statistical Package for Social Sciences) was used for univariate and bivariate analysis. In univariate analysis, such as frequency, percentage and measures of

central tendency (mean, standard deviation) were used to explain the data. In bivariate analysis, association amongst diverse variables was examined all the way through applying chi-square test at the 0.05 percent level of significance. The positive and negative relationship of variables was checked by the gamma test. Bad personality development is taken as dependent variable and it is operationalized through a number of reactions (low, medium, high) to various statements.

Table 1: Association between demographic variables and bad personality development

*Dependent Variable: Bad personality development				
Independent Variables (Demographic)	Chi-square statistics		Gamma statistics	
	Value	Sig. Level	Value	Sig. Level
Age (10-14, 15-19)	19.531	0.000	0.447	0.000
Family type (nuclear, joint)	43.812	0.000	0.520	0.000
Sibling size (up to 3, 4-6, 7 or above, not at all)	21.928	0.001	0.290	0.000

The researcher used the technique of data analysis in which two variables are examined simultaneously in association with one another. The association between a dependent variable (bad personality development) along with independent variables: demographic variables (age, family type, sibling size) were carried out. The chi-square and gamma statistics were used to check the association and testing hypotheses.

In the light of the results, the chi-square value (19.531) indicated a highly significant association between age of the respondents and bad effect on personality development; whereas, the gamma value (0.447) verified a strong positive relationship. It means that as age increased the bad effects on personality development also increased, so the hypothesis of an association between higher age and bad effect on personality development is accepted. The parental conflict was associated with internalizing and externalizing problems among adolescents. The chi-square value (43.812) and gamma value (0.520) confirmed a strong positive relationship between joint family structure and bad effect of personality development. Results shows that the hypothesis of an association between family type and bad effect on personality development is accepted. The joint family framework is very regular in Pakistan while in nuclear families, children were closer to their parents and more franked discussing about their problems, which helped in the better development of the personality. The value of chi-square (21.928) shown a significant association between a large number of siblings and bad effect on personality development. The value of gamma (0.290) shown a strong positive association between the variables. It means those respondents who had a large number of siblings had highly bad effect on their personality development. Large sibling size have bring down subjective capacities and expanded behavioral issues in the family. In this way the hypothesis of an association between the higher number of sibling and bad effect on personality development is accepted.

Table 2: Association between cultural variables of parental conflict and bad personality development

Dependent Variable: Bad personality development				
Independent Variables (Cultural)	Chi-square statistics		Gamma statistics	
	Value	Sig. Level	Value	Sig. Level
Reasons of conflict (financial problem, dowry, relatives or in-laws, demand for baby boy, inheritance, just had a bad day, not happy together, children, joint family system, psychological disorder, misunderstanding, father spent most of the time outside the home, infertility)	47.429	0.000	0.388	0.000
Extent of inter-parental conflict (Get mad when argue, usually work it out when have an argument, stay mad at each other when stop arguing, parents discuss quietly their disagreements, parents say mean things to each other, parents broke or throws things, parents pushed or shoved each other, parents have a smooth communication)	39.768	0.000	0.451	0.000
Drug addiction (Ghutka, naswar, sleeping pills, heroin, hookah or shisha, cigarette, alcohol, pan, supari)	60.984	0.000	0.599	0.000
Health problems (skin disease, obesity, body pain, depression, headache, fever, asthma, whooping cough, underweight, shivering in body, psychological disorder, physical disability, dizziness, sexual health problem, speech disorder)	35.812	0.000	0.426	0.000

The association between a dependent variable (bad personality development) along with independent variables: cultural variables (reasons of conflict, extent of conflict, drug addiction, health problems) were carried out. The chi-square and gamma statistics were used to check the association and testing hypotheses.

Parental clash is poisonous for adolescents. Children who are exposed to parental conflict do not interact well with others. These children frequently have extremely poor social attitudes, low confidence and poor connections when they progress toward becoming grownups. The value of chi-square (47.429) shown a highly significant (P = 0.000) association between reasons of conflict and bad effect on personality development. The gamma value (0.388) also shown a positive relationship between higher reasons of parental conflict and higher bad effect on adolescent personality development, so the hypothesis of an association

between reasons of inter-parental conflict and awful effect on the development of the personality is accepted. The value of chi-square (39.768) shown a highly significant association (P = 0.000) between extent of parental conflict and bad effect on adolescent personality development. The gamma value (0.451) also shown a strong positive relationship between the variables. The hypothesis of a connection between the level of inter-parental conflict and bad outcome in the development of the personality is accepted. The impact of inter-parental clash on youngsters depended both upon the way in which it is communicated, overseen and settled, and also the degree to which adolescents feel to blame for, or undermined by, their parent's relationship contentions.

The value of chi-square (60.984) and the value of gamma (0.599) confirmed a strong positive association between drug addiction and bad effect on personality development, so the hypothesis of a connection linking drug addiction and bad effect on personality development is accepted. Adolescents who disliked their emotionally denied homes and reluctant to express their anger, utilized drugs as one approach to dealing with their stifled anger. The value of chi-square (35.812) shown a highly significant association (P = 0.000) between health problems and bad effects on personality development. The gamma value (0.426) demonstrated a strong positive relationship between the variables. The hypothesis of an association between health problem and bad effect on personality development is accepted. It means parental conflict damaging children who were in the stage of physical and emotional development. Parental conflict was the cause of creating depression among adolescents. The threatening relationship of the guardians in Pakistan was at raising hazard for mental and physical medical issues. These results also supported by the theoretical models of the research.

CONCLUSION

Based on survey results, it is concluded that adolescents who faced parental conflicts have more bad effects of their personality development. In developing countries like Pakistan, the parental conflict considers not a big issue. It is the story of every home. The family structure in any country depends upon its culture and living style. In Pakistan, individuals see and trust that the capacity of the family is to deliver and acculturates and mingle children. There is a strong hierarchical system. Management and decision-making issues in the joint family largely arose due to the failure to face up to, or communicate with, head person within the family, thus leading to disappointment and passive hostility. Children from the joint family has more conflicts, less compromised because of their socioeconomic status where resources are limited. Guardians' limited levels of assets (time, cash, energy, etc.) weakened among children as the number of siblings increases. Parental conflict results in reduced parental involvement, harsh discipline practices, lack of praise and acknowledgement, and increased parent-child conflict. The parenting behavior of the parents also badly affected by inter-parental conflicts. Continuous parental conflict affects parents' emotional health and parent-child relationship. Parental attitudes and approaches impact children. Children may experience angst, embarrassment, despair, or other issues when conflicted parental relationships result in dysfunctional parenting practices. Stress hormones have the bothersome impact of empowering addiction, as they influence those areas of the mind that the reason the body to ache for a greater amount of the drugs. The more children are exposed to conflict, the more sensitive they become to its side effects. The level of inter-parental conflict is strongly related to the bad effects on the development of the adolescent's personality.

RECOMMENDATIONS

Based on the findings of the research, it is recommended that:

1. The state should make sure adolescent rights (e.g., wellbeing facilities) are regarded, secured, and fulfilled. This incorporates helping families ensure adolescent rights, and make a situation where children can develop to their maximum capacity.
2. There should be a psychologist or counselor to secure children's personality from the bad effects of parental conflicts.
3. Parents should realize that their adolescents are hurt less by divorce than by experiencing the severe conflict, whether their families are intact, dissolving, or wrecked.
4. Never reprimand adolescent for whining, crying or staying aloof. Parents should try to find out the reason behind the unnatural behavior.

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Ethnomedicinal Uses of the Plants of Tehsil Laalqilla, District Lower Dir, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Tehsil Laalqilla of District Lower Dir, Khyber Pakhtunkhwa, Pakistan has a diverse flora of medicinal plants. 50 Medicinal taxa were evaluated which were distributed in 47 genera and 34 families. Amongst them one taxa was Monocotyledonous (2.0%) while remaining forty nine taxa was Dicotyledonous (98.0%). Plants were mostly used for various ethno medicinal purposes viz. Abdominal Pain, Diarrhea, Dysentery, Diuretic, Blood Purifier, Refrigerant, Tonic, Antispasmodic, Analgesic, Toothache, Healing of wounds, Skin diseases and Kidney stones.
KEYWORDS: Ethno medicinal; Laalqilla; Lower Dir; Khyber Pakhtunkhwa; Pakistan.

INTRODUCTION

Tehsil Laalqilla of District Lower Dir is located between 34°-37' to 35°-07' North latitude and 71°-31' to 72°-14' East longitude in Khyber Pakhtunkhwa, Pakistan. Tehsil Laalqilla is connected towards north with Upper Dir district and shares its boundaries towards East with Swat district, towards south connected with Malakand district and connected with Afghanistan and Bajour agency on west side. Its altitude ranges from 1300 to 2000 meters having hilly and mountainous areas which are covered with snow during winter season. The temperature rises from May to august and decreases from December to February. Mostly rainfall and snowfall occurs in winter season while summer is moderate. The area is occupied by the forests of *Pinus, Taxus, Abes, Quercus, Accacia, Olea* etc. The major crops grown are *Triticum aestivum, Zea mays, Oryza sativa, Hordeum vulgare, Solanum tuberosum, Lycopersicon esculentum, Brassica campestris, cucurbita maxima* etc. Amongst the fruits grown in the area are *Malus pumila, Prunus persica, Psidum guvava, Citrus medica, Juglens regia and Diosporus lotus* etc [1]. The plants which contains active bio chemicals constituent and having some response in the curing of disorders in living organisms are known as medicinal plants [2]. Pakistan has a diverse flora having 6000 species of Flowering Plants. Amongst them 700 plant species are used for medicinal purpose [3]**.** In Pakistan 80% of the people belonging to the rural areas and still depends upon the herbal medicines. The herbal medicines occupy distinct position right from the primitive period to present day. The ethno medicinal history of Plants is as old as man himself [4]. These medicines can be get easily from the nature and have fewer side effects. Pakistani people basically prefer Unani system but the ethno medicinal plants use is also seen in the remote areas [5]. In developing countries especially in those areas which have insufficient approach to modern medicines medicinal plants are used by local peoples [6]. Medicinal plants belong to group of plants that contain distinctive chemical compound in their body and are applied for diverse purposes [7]. Due to the unavailability of allopathic doctors and modern medicines in the remote areas and due to fears from the side effects of modern medicines local peoples prefers traditional system of medicine [8]. New plant species have been recorded for the flora of Pakistan which has great medicinal values [9]. Various plant hormones are also used by the local peoples to improve the yield and productivity of food crops [10]. The people of this remote area also have no alternative beside to follow the old tradition due to the less number of physicians. Therefore the objective of the study was to document plants of the remote areas of district Lower Dir, Khyber Pakhtunkhwa, Pakistan which were used as medicine by the local and tribal population.

MATERIALS AND METHOD

The research was conducted during January to May 2017 in tehsil Laalqilla, district Lower Dir, Khyber Pakhtunkhwa, Pakistan. Field trips were arranged for the collection of plants the collected specimens were tagged, pressed, poisoned and pasted on standard herbarium sheets for proper identification. The plants were identified with

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the help of available literature [11]. The valuable information and indigenous knowledge was documented about the uses of ethno medicinal plants from the stakeholder of the local areas through questionnaire and interviews. Questionnaire was used for the documentation of indigenous knowledge and questions were asked from local people viz. Hakims, wound healers, farmers and old women's.

Table 1. Ethnomedicinal Plants of District Lower Dir, Khyber Pakhtunkhwa, Pakistan.

Sr.No	Botanical name	Local Name	Family	Part/s used	Habit	Method of uses
1	*Acacia modesta* Wall.	Palusa	Mimosaceae	Gum	Tree	Gums used for binding of tablets in capsules covering.
2	*Achyranthus aspera* L.	Geshy	Amaranthaceae	Leaves	Herb	Decoction of leaves used for toothache.
3	*Ajuga Bracteosa* Wall.ex Benth.	Gooti	Lamiaceae	Leaves	Herb	Leaves increase passage of urine from urinary tract, tonic, relives stomach and abdominal pain.
4	*Allium cepa* L.	Peyaz	Liliaceae	Bulb	Herb	Bulb is carminative and used in obesity.
5	*Amaranthus virids* L.	Ganhar	Amaranthaceae	Leaves	Herb	Leaves used for softening of stomach.
6	*Ammi visnaga* Lamk.	Spirkai	Apicaeae	Seeds	Herb	Seeds useful for intestinal and stomach disorders.
7	*Artemisia maritime* L.	Tharkha	Asteraceae	whole plant	Shrub	Used as anthelmintics to remove worms from intestinal tract.
8	*Berberis Lycium* L.	Kwary	Berberidaceae	Roots	Shrub	Roots used in Hepatitis A, cooling agent, healing of wounds and relieve pain of vertebral column.
9	*Berberis Vulgaris* L.	Kwary	Berberidaceae	Roots	Shrub	Roots regulate fever, relieves pain of vertebral column.
10	*Brassica campestris* L.	Sharsham	Brassicaeae	Oil	Herb	Oil is best cooling agent of stomach for animals.
11	*Calotropis Procerea* L.	Spulmai	Asclepidiaceae	Latex, Leaves	Shrub	Leaves used in cholera, asthma & dental cleaning. Latex is used against snake biting.
12	*Carthamus oxycantha* Bieb.	Kareeza	Asteraceae	Oil	Herb	Oil is used as a tonic.
13	*Chenopodium album* L.	Sarmay	Chenopodiaceae	Seeds	Herb	Seeds are anthelmintic, soften the stools and expel them.
14	*Citrus medica* L.	Lambo	Rutaceae	Fruit	Tree	Contains citric acid used to remove prickles of face, makes face soft and cause healthy hair growth.
15	*Coriandrium sativum* L.	Dania	Apicaeae	Seeds	Herb	Seeds are carminative contains volatile oils which used as a flavoring agent.
16	*Daphne mucronate* Royle.	Laighonai	Urticaceae	Fruits	Shrub	Fruits used to prevent loose motion and vomiting.
17	*Datura innoxia* Mill.	Baturra	Solanaceae	Leaves, seeds	Shrub	Leaves extract used as toothache, epilepsy and headache, treatment of swollen limbs. Seeds are antipyretic and narcotic.
18	*Datura stramonium* L.	Baturra	Solanaceae	Seeds	Shrub	Seeds contain alkaloids which are anodyne viz. relives pain from the body.
19	*Dodonea viscosa* L.	Ghurasky	Sapindaceae	Leaves	Shrub	Leaves used as astringent and in gas trouble.
20	*Eruca sativa* Mill.	Jamama	Brassicaeae	Seeds	Herb	Seeds are stimulant and prevent irritation of eyes.
21	*Eucalyptus camaldulensis* Deh.	Lachi	Myrtaceae	Leaves	Tree	Oil obtained from leaves is anti asthmatic and antiseptic.
22	*Euphorbia heloiscopia* L.	Mandaro	Euphorbiaceae	Roots	Herb	Roots are purgative, relieves chronic coughing and dysentery.
23	*Ficus carica* L.	Inzar	Moraceae	Fruit	Tree	Fruits contain minerals and are a tonic.
24	*Foeniculum vulgare* Mill.	Kaga	Apiaceae	Seeds	Herb	Seeds are stimulant and carminative.
25	*Fumaria indica* Hausskn.	Shatara	Fumariaceae	Whole plant	Herb	Juice used to prevent ulcer, fever, increase the passage of urine from urinary bladder.
26	*Geranium wallichianum* D.Don ex Sweet.	Sor booti	Geraniaceae	Rhizome	Herb	Rhizome used to prevent vaginal problems in females.
27	*Hedera nepalensis* K.Koch.	Parvatha	Hederraceae	Leaves	Herb	Leaves used to make stools normal and person does not feel difficulty during expelling it.
28	*Juglans regia* L.	Akoor	Juglandaceae	Fruit, Bark	Tree	Fruits are rich in proteins used for cleaning of teeth and throat infections. Bark used in dry cough.
29	*Justicia adhatoda* L.	Baikar	Acanthaceae	Leaves	Shrub	Leaves used in bronchitis, cough, and prevents loose motion.
30	*Melia azedarach* L.	Shandai	Meliaceae	Leaves, seeds	Tree	Leaves used in diabetes and seeds in chest infections.

31	*Mentha arvensis* L.	Podina	Lamiaceae	Leaves	Herb	Leaves boiled in green tea for vomiting and nausea.
32	*Mentha longifolia* L.	Velany	Lamiaceae	Leaves	Herb	Contains high percentage of volatile oils and is best carminative.
33	*Micromeria biflora* Benth.	Kashmale	Lamiaceae	Leaves	Shrub	Leaves used for relieving pain from facial regions.
34	*Nerium odorum* Soland.	Ganderi	Apocynaceae	Leaves	Shrub	Leaves are analgesic, prevents bleeding gums and give strength to teeth.
35	*Ocimum basilicum* L.	Kasmali	Lamiaceae	Leaves	Herb	Leaves juice used in cough and ear infections.
36	*Olea ferruginea* Royle.	Khona	Oleaceae	Seeds	Tree	Oil of seeds is used in rheumatic joints.
37	*Oxalis carniculata*	Threwaky	Oxalidaceae	Whole Plant	Herb	Prevent stomach pain, acidity, remove worms from intestine.
38	*Papaver somniferum* L.	Doda	Papaveraceae	Seeds, latex	Herb	Before the discovery of chloroform it was used as anesthetic as a surgery. But now used to relive severe pain.
39	*Plantago lanceolate* L.	Satt	Plantaginaceae	Seeds	Herb	Used to feel easy in the removal of bowls from large intestine.
40	*Platanus oriantalis*	Chinar	Plantanaceae	Bark	Tree	Bark used in joint ache and prevents loose motion.
41	*Podophylum emoidi* Wall.	Kakora	Phodophyllaceae	Root	Herb	Roots relieve pain from the body.
42	*Punica granatum* L.	Anaar	Punicaeae	Pulp	Shrub	Pulp used for the treatment of constipation.
43	*Quercus incana* Roxb.	Serray	Fagaceae	Bark	Tree	Bark used in kidney disorders, increase the passage of urine and controls diabetes.
44	*Ricinus communis* L.	Aranda	Euphorbiaceae	Seeds	Shrub	Seeds used to prevent loose motion.
45	*Solanum nigrum* L.	Kachmacho	Solanaceae	Leaves, Seeds	Herb	Leaves are sedative, epitizer, purgative, diuretic and expectorant. Berries are tonic and effective in hepatitis.
46	*Thymus linearis*	Spairkai	Lamiaceae	Fruit	Herb	Used in fever, cough cold and stomach disorders.
47	*Viola serpens* wall.	Binfsha	Violaceae	Leaves	Herb	Juice of leaves used in wounds and joints disorders.
48	*Xanthium strumarium* L.	Geeshy	Asteraceae	Leaves	Herb	Leaves chewed for dental infections viz. dental decay.
49	*Zizyphus sativa* Gaethn.	Beera	Rhammaceae	Leaves	Shrub	Leaves used to regulate fever, prevents flow of blood in stools.
50	*Zanthoxylum armatum* DC.	Dambara	Rutaceae	Seeds	Shrub	Seeds used in fever and cholera.

RESULTS AND DISCUSSION

A total of 50 taxa were evaluated which were distributed in 47 genera and 34 families (table 1: fig 1). Out of them one taxa were Monocotyledonous while remaining forty nine taxa were Dicotyledonous. Habit wise twenty seven taxa were herbs; fourteen taxa were Shrubs while remaining nine taxa were Trees used by the local peoples for the treatment of various ailments (fig 2). Lamiaceae were the largest family having six taxa followed by Apiaceae, Asteraceae and Solanaceae having three taxa each. Amaranthaceae, Berberidaceae, Brassicaceae, Euphorbiaceae and Rutaceae have two taxa each used for different disorders while the remaining families viz. Mimosaceae, Lilaceae, Asclepidaceae, Chenopodiaceae, Urticeae, Sapindaceae, Myrtaceae, Moraceae, Fumariaceae, Geraniaceae, Hederraceae, Juglandaceae, Acanthaceae, Meliaceae, Oleaceae, Apocynaceae, Oxalidaceae, Papaveraceae, Plantaganaceae, Plantanaceae, Phodophyllaceae, Puniceae, Fagaceae, Violaceae and Rhamnaceae have one taxa each. All these plants were wild and used by local peoples for various ethno medicinal purposes viz. Abdominal Pain, Diarrhea, Dysentery, Diuretic, Blood Purifier, Refrigerant, Tonic, Antispasmodic, Analgesic, Toothache, Wounds healing and skin diseases.

1. The similar study was previously conducted from Hazar Nao forest, Dargai, district Malakand, Khyber Pakhtunkhwa, Pakistan [12], from Malam Jaba, district Swat, Khyber Pakhtunkhwa, Pakistan [13], Dir Kohistan, district Upper Dir, Khyber Pakhtunkhwa, Pakistan [14], from Charkotli Hills, Batkhela district Malakand, Pakistan [15], Chitral district, Khyber Pakhtunkhwa, Pakistan [16], from Shawar valley, district Swat, Khyber Pkhtunkhwa, Pakistan [17], from Samarbagh valley district Lower Dir, Khyber Pakhtunkhwa, Pakistan [18], checklist of medicinal plants of district Lower Dir, Khyber Pakhtunkhwa, Pakistan [19], checklist of the pteridophytes of district Mansehra, Khyber Pakhtunkhwa, Pakistan [20],

from Maidan valley district lower Dir Khyber Pakhtunkhwa, Pakistan [21], from Dilbori (OGHI), District Mansehra, Khyber Pakhtunkhwa, Pakistan [22], from Upper Tanawal, District Mansehra, Khyber Pakhtunkhwa, Pakistan [23] from District Tor Ghar, Khyber Pakhtunkhwa, Pakistan [24], from Kaghan Valley, District, Mansehra, Khyber Pakhtunkhwa, Pakistan [25] and from South of Chad similar study was reported [26]. The present study would be helpful for other researchers who are interested in Pharmaceutical study.

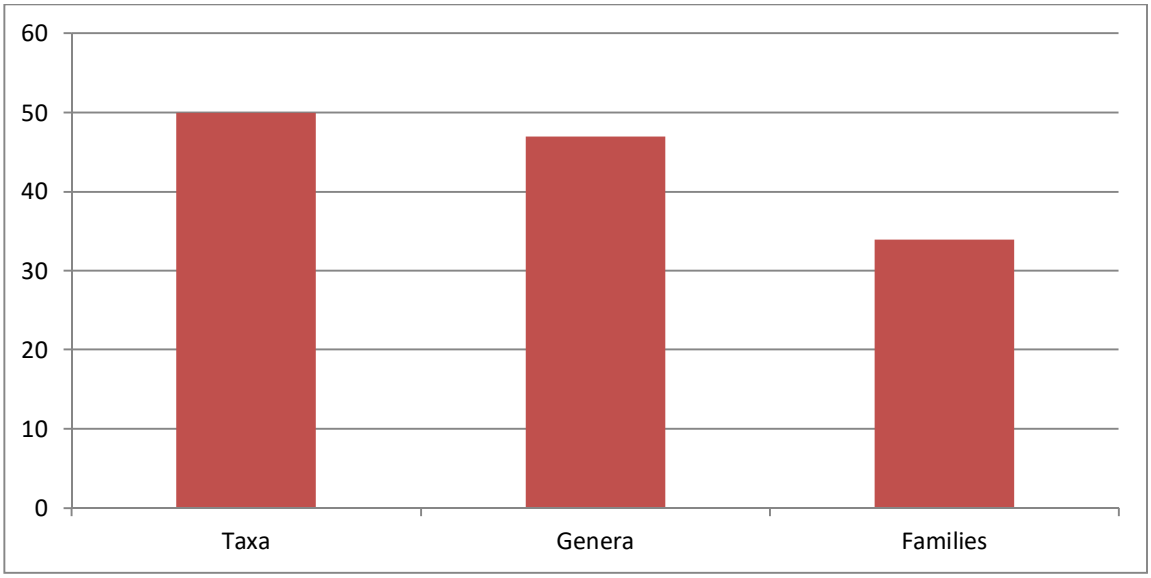

Fig: 1. Ethnomedicinal uses of the number of taxa, genera and families of tehsil Laalqilla, district Lower Dir, Khyber Pakhtunkhwa, Pakistan.

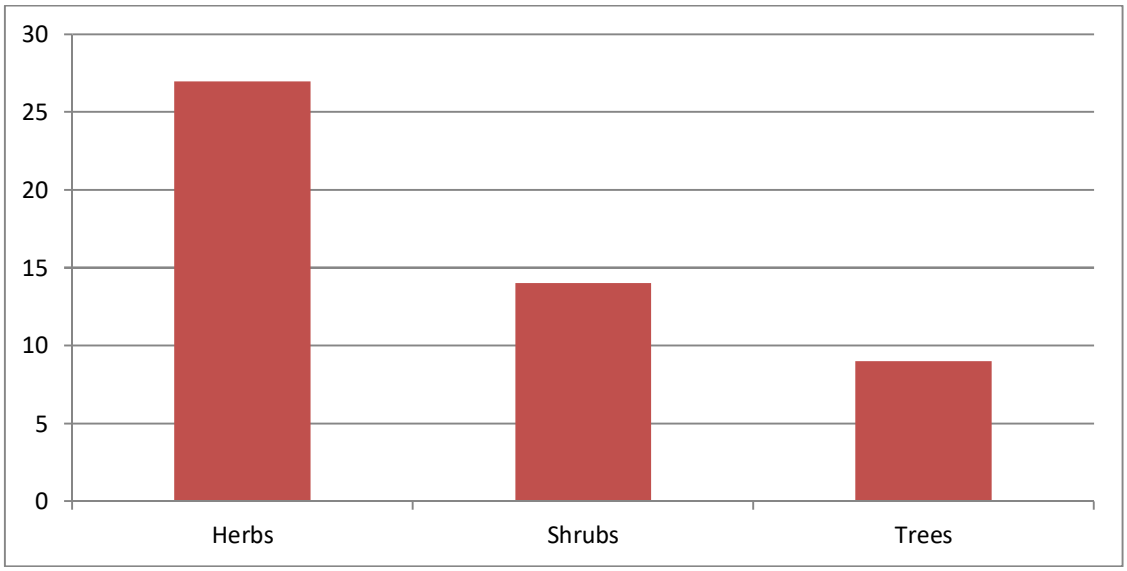

Fig: 2. Ethnomedicinal uses of herbs, shrubs and trees of tehsil Laalqilla, district Lower Dir, Khyber Pakhtunkhwa, Pakistan.

CONCLUSION

The recent study concluded that most of the population of tehsil Laalqilla, district Lower Dir, Khyber Pakhtunkhwa, Pakistan were involved in the collection and using of these medicinal taxa. Mostly inhabitants of hilly, tribal and far-flung areas were involved in their collection. Knowledge-wise aged people and poor people have more knowledge about the uses of these medicinal taxa. Mostly the plant taxa were used against disorders viz. hepatitis, diabetes, fever, ringworm, hair-care, asthma, bronchitis, pneumonia, emphysema, kidney stones, sterility and tonic. These taxa can be explored for new drugs discovery and exploited commercially keeping in view their availability. Cultivation and conservation of these taxa for different purposes is strictly needed.

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Conflict of interest:
There is no conflict of interest amongst the authors.

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Correlation between ABO Blood Groups and Proficiency in Computer Gaming

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ABSTRACT

In Human blood group systems, the ABO blood group is a most important system. Numerous studies have analyzed the possible correlation between ABO blood groups and different diseases and personality traits. This article examined the probable correlation between ABO blood groups and proficiency in computer gaming. From April 20017 to November 2017, a total of 146 subjects was analyzed. The results of a present study indicate a statistically significant correlation and suggest that individuals with group O have more proficiency in computer gaming. Female are less proficient in computer gaming than males. The correlation between ABO blood type and computer gaming is therefore suggested by this study.
KEYWORDS: Blood Groups, ABO blood grouping, Computer Games.

1. INTRODUCTION

Blood is a body fluid in humans and animals that carries elementary substances like oxygen and nutrients to the body cells and move metabolic wastage away from these cells. Plasma, red blood cells, white blood cells and platelets are the elementary components that comprise human blood. Blood is indispensable to life as there is no potential alternate to blood.
The human blood group system is a mechanism that categorized the blood on the presence or absence of particular markers. According to International Society of Blood Transfusion, 36 human blood group systems have been identified [1]. ABO is one of a common blood grouping system discovered by Karl Landsteiner in 1901. In ABO system, the classification of human blood is grounded on congenital properties of red blood cells as defined by the absence or presence of the antigens. The antigens belong to ABO blood group are associated to oligosaccharide chains at the surface of the erythrocyte membrane, and also on the surface of many other kinds of cell types like endothelia and epithelia. ABO gene is located at the band 9q34.2 and includes 7 exons. The ABO gene encodes three allele forms: A, B and O, which eventually combine to form four major ABO blood groups in humans (i.e., A, B, AB and O). Two antigens and two antibodies are mostly in authority for the ABO types. Table 1 describes the possible permutations of antigens and antibodies with the corresponding ABO blood type.

Table 1. Combination of Antigens and Antibodies in ABO Blood

ABO Blood Type	Antigen (A)	Antigen (B)	Antibody (anti-A)	Antibody (anti-B)
A	Yes	No	No	Yes
B	No	Yes	Yes	No
AB	Yes	Yes	No	No
O	No	No	Yes	Yes

The medical importance of the ABO blood group system expands beyond the transfusion and transplantation sciences and numerous reports have reported on the imperative association in the development of oncological, cardiovascular and other issues [2,3]. ABO blood types are not restricted to human, but also presents in animals like chimpanzees and gorillas [4].
Video games are the digital games and a kind of interactive multimedia and generally used for entertainment. Computer games are existed since the introduction of computing technology, and gain popularity and diversity with the expansion of computing technology. It was normally postulated that computer gaming has negative

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consequences on young players, yet petite evidence has appeared to support these claims. The computer games were initially developed for entertainment, yet in recent eras there has been an increase of concentration in the use of games for learning and behavioral variation [5].
Durkin and Barber in [6] reported a study which analyzed the relation between game playing and different measures of adjustment or risk taking on students. The study identified no negative impact on game players. The study resolved that computer games can be a constructive feature of a healthy adolescence.
Sylvén and Sundqvist [7], described that multiplayer online game delivers L2 English beginners with linguistically substantial and cognitively thought-provoking environment that may be helpful to L2 learning, because learner acquire different prospects for the input and interaction in the L2. Similarly, Kebritchi et al. [8], identified the impact of a computer game on students' mathematics accomplishment in public high school setting.
SimCity is a game developed for the simulation of city-building and urban planning. The game is available for multiple platforms and used the GlassBox as an engine. It allows the players to develop a settlement that may expand into a city by zoning land for industrial, commercial or residential development. Kim and Shin [9] analyzed the pedagogical benefits of SimCity and described that the use of SimCity can be a useful tool for learning geography.
In [10], the impact of using digital game and its role in increasing children's vocabulary learning was examined. In the treatment group, the SHAIEx game was used, whereas the traditional method was used in the control group to teach the English vocabulary. The study revealed that the use of digital game has a positive impact on teaching since the mean score of the subject in the treatment group was higher than those in a control group.
In [11], the effect of inference-based computer games on Chinese students studying English was investigated. The study reported that subjects learned more vocabulary in computer game condition than in the condition that follow the conventional study method.
The existing studies on the impact of using computer games in different discipline has shown mild results, but the reason of proficiency in gaming is still a less addressed area. The blood group has a strong association with different personality traits and medical diseases and therefore a decisive study is conducted which is based on the research question that whether a particular blood group affects the proficiency in computer gaming. The rationale of the study is to eliminate the gap in the literature related with elucidation of traits that affects the proficiency in computer gaming.
The trend of using computer games to support different areas has been increasing very rapidly but the varying level of proficiency in gaming has found in the gamer. This problem is virtually addressed in this article and the reported study would be significant in analyzing whether antigens and antibodies possibly affects the proficiency of computer games.
The rest of the paper is organized as follows. The related work is described in section 2 and the design & methods are included in section 3. Discussion is included in section 4. Finally, section 5 described the conclusion.

2. RELATED WORK

Several notable studies have been conducted to analyze the possible relationship between the blood groups and different diseases and capabilities.
In [12], 150 medical students are studied and their probable association between blood groups and bleeding time and clotting time are identified. The study reported that O blood group individuals have greater bleeding time and clotting time than the non-O blood group individuals. The study also observed that males had less bleeding time and clotting time than females.
Agari [13], analyzed the effect of blood groups on group participation. The study reported that subjects blood groups have an impact of their group cooperation and the A blood group holders have the highest inclination to group participation, followed by O, AB, B respectively. Similarly, in another study [14], the same kind of results is identified. The study identified that there is a positive and significant association between ABO blood groups and group participation, and the individual having A blood group has the highest group participations, and followed by O, AB and B respectively.
In [15], 1427 healthy Japanese subjects were analyzed and the association between the ABO genotypes or ABO phenotypes and personality traits were statistically examined by using multivariate analysis of covariance. The study identified a significant association between ABO group genotypes and personality traits on the analyzed subjects.
A study reported in [16] confirms the existence of a correlation of ABO blood groups with cholesterol level and coronary heart disease coronary artery disease and myocardial infarction. The study was conducted on 6476 Chinese subjects undergoing coronary angiography.
Iranfar and Letard [17] conducted a correlational study to elucidate the association between ABO blood groups and hypnotic susceptibility. The modified Hypnotic susceptibility form is used on a tiny sample and result identified that there is a strong correlation between ABO blood groups and hypnosis.
Gupta [18], explore the correlation between ABO blood groups and emotional intelligence by the analysis of 200 college students. The study indicated that individuals having AB+ blood group are higher on emotional stability and empathy, whereas the individuals having B+ blood group are higher on value orientation.

Atoom in [19], analyzed the association between ABO blood groups and the intelligence. During the study, 364 students of universities were tested. The study identified that AB blood group has the highest average in Intelligence Quotient test, whereas the B blood group has the lowest performance in the test results. In another study [20], the association between ABO blood groups and Intelligence Quotient in a particular region is identified. During the study, 263 subjects from rural area and 367 subjects from the urban area were selected. The study identified that in rural areas, males with blood group O have a high IQ level and similarly the females with a blood groups O have a high IQ level and the females with blood group B have low IQ level. In urban areas, the male subjects with O blood group have high IQ level and followed by the B blood group.

Running performance is known as a key driver of evolutionary benefit in humans, and keeping this view, Lippi et al. [21], analyzed whether the ABO blood groups have any effect on running performance. During the study, 52 middle-age athletes who are recurrently involved in endurance activity were selected. The study revealed that athletes with O blood group have better endurance as compare to the athletes with other blood groups.

Tuberculosis is a disease caused by Mycobacterium tuberculosis and affects the lungs. In [22], the association between ABO blood and pulmonary tuberculosis is examined on a particular geographical region. In the study, the treatment group comprised of 122 cases and control group comprised of 2842 cases. The study reported that A and B blood group individuals are more expected to suffer tuberculosis as compared to individuals with O and AB blood groups.

In [23], the possible correlation between ABO blood groups with blood pressure is analyzed. During study the 835 students were analyzed. The study identified that the individuals with A blood group have a high risk of heart diseases compared to other blood groups.

There are many other notable studies that investigated the impact of blood group type with personal traits and medical diseases. For more detail, see [24-28].

3. DESIGN & METHODS

In [29], Bleakley et al. reported a meticulous study conducted in the examination of literature on computer games and serious games in connection to the possible effects of gaming on the users, particularly on learning, skill enrichment and engagement. The study identified that playing computer games is associated with an array of cognitive, perceptual, social, affective and motivational effects and consequences. All these are naturally associated with the biological state of human and obviously with the blood group.

In order to explore the existing knowledge on the influence of ABO blood group system, a tiny study has conducted to identify the probable association between ABO blood groups and the proficiency in playing computer games.

This study population consisted of 146 recreational, young adults, computer literates (mean age: 22 ± 2 years), who normally play the computer games. The subjects for the study were selected by using stratified random sampling from the strata based on the ABO blood groups and categorized in four groups according to the blood groups of subjects. During the study it was endeavored to equalize the number of subjects (participants) in each blood group, yet the subjects in AB group are lesser than the other groups. The number of subjects in each group and their percentage in the total is shown in Fig. 1.

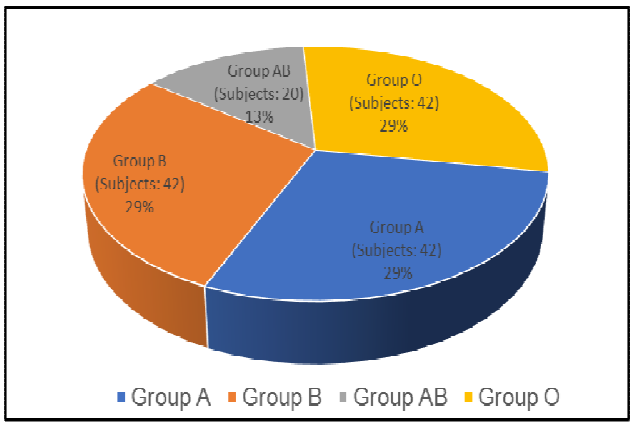

Fig. 1: Percentage Distribution of ABO in the Study

In three groups (A, B and O) the number of subjects was equal, whereas the AB group had a comparatively less number of subjects. Table 2 illustrates the detail of male and female participants included in the study groups.

Table 2. Detail of Subjects Participated in the Study

Subjects	Blood Groups			
	A	B	AB	O
Male	21	21	12	21
Female	21	21	8	21
Total	21	21	20	42

The study was started in April 2017 and completed in November 2017. All subjects voluntarily participated in the game. *Dave* is a one of a classic computer game and primarily used in a study to examine the possible association. This game is merely selected due to its simplicity and a fact that none of any subject in the study was acquainted with this game. Before examining all the stages and strategies of the game were practically demonstrated to all the participants and each participant was allowed to play the game three times before the formal evaluation. The results of formal evaluation are shown Table 3.

Table 3. Results of Formal Evaluation

Blood Groups	Win	Lose
A	13	29
B	17	25
AB	5	15
O	27	15

The winning percentage of four groups in the study were: 30.95% for group A, 40.48% in group B, 25% in group AB and 64.29% in group O, which indicates that winning ratio of O group is much higher than the other groups. For more and detailed analysis, a Chi-square test was conducted on the competency (win or lose) of the subjects in computer gaming. The results are shown in Table 6.

Table 4. Result of Chi-Square test

Test	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.028	3	.005
Likelihood Ratio	13.167	3	.004

As can be seen by the statistics tabulated in Table 4, there is a significant relationship between ABO blood groups and competency in computer gaming, $\chi^2(3, N=146) = 13.03$, $p < .05$.
To simplify the statistical analysis the score secured by participants in formal investigation are coded into the equivalent numbers ranged from zero to hundred and illustrated in Fig. 2.

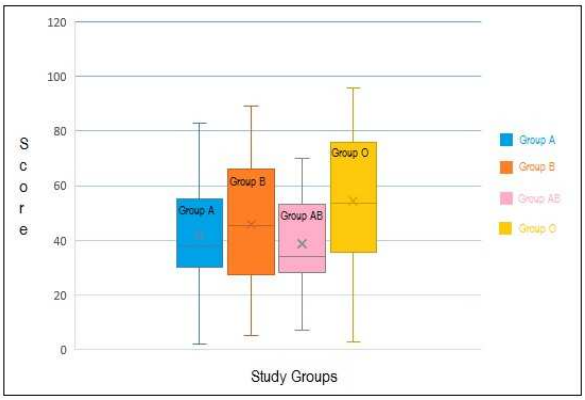

Fig. 2: Boxplot of score

The upper whisker and upper quartile of boxplots indicates that individuals in group O have secured more marks than the other groups. One-way ANOVA test is conducted on the coded score of the subjects in each group and descriptive results are shown Table 5.

Table 5. Descriptive Statistics of one-way ANOVA Test on the score of four groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	42	41.95	19.283	2.976	35.94	47.96	2	83
B	42	45.79	22.003	3.395	38.93	52.64	5	89
AB	20	38.80	19.256	4.306	29.79	47.81	7	70
O	42	54.50	24.511	3.782	46.86	62.14	3	96
Total	146	46.23	22.210	1.838	42.60	49.87	2	96

Among all groups the group O has highest mean score (54.50), and the subject with the highest score (96) also belonged to the blood group O. The result of one-way ANOVA showed that there was a statistically significant effect of ABO blood groups on proficiency in computer gaming remembered at the $p < .05$ for the conditions [(3, 142) = 3.370, p = .020].

The highest score in a game was secured by a male subject and therefore the proficiency of male subjects in all groups is evaluated by a one-way ANOVA test and the descriptive statistics are shown in Figure 6.

Table 6. Descriptive Statistics of one-way ANOVA Test on the score of male subjects of four groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	21	46.57	20.191	4.406	37.38	55.76	7	83
B	21	47.38	22.515	4.913	37.13	57.63	13	89
AB	12	43.00	19.278	5.565	30.75	55.25	7	70
O	21	68.19	18.343	4.003	59.84	76.54	32	96
Total	75	52.28	22.254	2.570	47.16	57.40	7	96

Among all groups the male subjects in group O have the highest mean score (54.50), followed by B, A and AB respectively. The result of one-way ANOVA showed that there was a statistically significant effect of ABO blood groups on the proficiency of male participants in computer gaming remembered at the $p < .05$ for the conditions [(3, 71) = 6.128, p = .001].

During study the difference between the performance of male and female subjects is analyzed with t-test by using the score of subjects in formal evaluation. The results are shown in Figure 7 and 8.

Table 7. Group statistics

Subjects	N	Mean	Std. Deviation	Std. Error Mean
Male	75	52.28	22.254	2.570
Female	71	39.85	20.438	2.426

The mean score of male participants (52.28) is much higher than the mean score of female participants (39.85), which indicates that male subjects are more proficient in computer games than the female subjects.

Table 8. T-test results

Assumption	Detail					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Equal variances assumed	3.511	144	.001	12.435	3.542	5.434	19.436
Equal variances not assumed	3.519	143.871	.001	12.435	3.534	5.450	19.419

The T-test conducted on the performance of subjects shows that there was a significant different score for male (M=52.28, SD=22.25) and female (M=39.85, SD=20.44) conditions; t(144)=3.51, p = .001.

4. DISCUSSION

The present study, evaluated the probable correlation between ABO blood types and proficiency in computer gaming. The total of 146 subjects (young adults) voluntarily participated in this study. The statistical significance of chi-square test conducted on the performance of individuals in gaming suggest that subjects with group O have better performance than the other groups. Similarly, the male subjects with group O have better performance than the other groups. The t-test conducted on the performance of male subjects revealed that female had less gaming proficiency in gaming than male. The reason for this vulnerability is not known.

On the whole the study implicitly suggests that the antigens and antibodies are not only related with blood cells and significant in blood transfusion, but they have many other biological and other significances and that need to be formally examined in further studies.

The finding of the current study may provide additional support to the putative evolutionary benefits of having the particular blood group. Moreover, the divergent kind of association in this article may motivate the other scholars to investigate the probable correlation of ABO blood groups and the comprehension of different domains like parsing [30] and programming [31-33].

5. CONCLUSION

The association between ABO blood type groups and human traits has no scientific consensus on the findings yet many studies statistically correlate ABO blood groups with different personality traits and medical diseases. In this article a study is reported that analyzed the probable association between the ABO blood group and proficiency in computer gaming on a small number of healthy subjects. The study suggested than individuals with blood group O have more proficiency in computer gaming and males are more proficient than females.

During study the subjects selected from Quetta, Pakistan were considered, so the finding of this article may not infer the subjects of other regions. The size of sample and the single region for the study are the main limitations of a study. To further expand the study in future, the following issues should be considered. First, it is fruitful to statistically analyze the impact of ABO blood on a large sample of different population. Second, various studies regarding the impact of ABO blood groups on human body and proficiency in computer gaming should be formally correlated by considering the biological aspects of antigens and antibodies. Third, the identification of other factors of human that may affect the proficiency of computer gaming should be considered. Fourth, correlating ABO blood groups with proficiency in computer gaming by allowing subjects to play different kinds of games in different environment. Sixth, the identification of academic, commercial, social and cognitive, pros and cons of proficiency in computer gaming.

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Perceptions of Teachers Regarding Causes of Absenteeism in Students at Secondary School Certificate Level in Baltistan

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ABSTRACT

Almost each and every country of the world has great concerns regarding absenteeism and finding ways to minimize or eradicate the phenomena. Absenteeism of students from schools is a serious issue, for all but specially in public sector. This study was conducted in Baltistan. Gilgit-Baltistan is one of the beautiful and charming places of the world which is situated in the northern side of Pakistan. Baltistan is known as one of the remotest regions of Islamic Republic of Pakistan. Due to its geographic location, basic life necessities are not available to its population as compared to other cities of Pakistan. Baltistan is going through the developing phase with meager resources. Educational facilities are also very limited for school going population. In this study surveys were conducted to obtain the data. All public sector secondary schools were included in population. Stratified sampling technique was adopted to determine the sample of the study. Specific questionnaire was constructed and data was collected from 02 teachers of each sample school. For analyzing the data both descriptive and inferential statistic was applied. Major findings of the study revealed that due to unawareness of parents about education is major cause of this issue. Lack of provision of guidance & counseling services to students was also contributing in absenteeism. Other findings showed that there was no provision of extra time& motivation for weak students, no incentives, and culture of punishment causing and increasing this phenomenon. One to base of findings and conclusions, it is recommended that awareness seminars for parents may be arranged. It was also recommended that community may also play its vital role to address this issue and to reduce absenteeism. Teachers may also be given special instructions and punishment should be completely prohibited.
KEY WORDS: Secondary Education, Absenteeism, Remote Areas, Baltistan

INTRODUCTION

Baltistan is one of the most beautiful areas of Pakistan. Geographically it is consisting of ten thousand one hundred and eighteen square miles mountainous area between the Karakorum and the Himalaya Ranges in the extreme north of Pakistan is called Baltistan. This consists of Skardu, Khaplu, Shigar, Kharmang, Rongyul and Gultari valleys. Kashmir is situated in south, Ladakh and Poreeg (Kargil) is in east, Gilgit and Diamir valleys are in the west of Baltistan whereas the Karakorum Range separates it from Xinxiang province of China in the west. People of Baltistan are civilize and friendly but slightly backward. Literacy rate is low. One of the major factors responsible for low literacy rate and backwardness is the fewer number of education institutions. Moreover people of Baltistan are not that much aware of the importance of education. To ensure that children get to school, it may be provided transportation at public expense and to ensure the tools of instruction when they get there the state may provide children with free text books and supplies [3]. For school community positive relationship to commune in the administration of secondary schools, there is needed to identify causes of absenteeism and lateness among secondary schools in Nigeria [4].

Baltistan is going through the developing phase with meager resources. Educational facilities are also very limited for school going population. The provision of these facilities i.e. availability of schools to the doorsteps of children has been addressed by public sector, private sector and NGOs as well. Attendance and retention of students is as necessary as provision of infrastructure, teachers and other facilities utilized in teaching learning process.

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In Baltistan region there are three well known sectors of educational institute are functional.

a. Aga Khan Education Service Pakistan (AKESP)
b. Private Education System
c. Government (Public) Education System

a. Aga Khan Education Service Pakistan (AKESP)

There are 126 AKESP schools and eight hundred and twenty two teachers to teach 22141 students in these schools. AKESP's mission is to "enable many generations of students to acquire both knowledge and essential spiritual wisdom needed to balance that knowledge and enable their lives to attain the highest fulfillment." AKESP is mainly focusing girls' education with some enrolment of boys in the co-education classes. AKESP continues to work with the government and communities to develop the models of public/private partnership that play a vital role towards implementing the Education Sector Reforms of the Ministry of Education, Government of Pakistan.

b. Private sector

Private sector has emerged as the second largest education provider in Gilgit-Baltistan. The need for private schools was felt as a result of people's dissatisfaction with the quality of education in the government schools. Some private schools were also opened to provide children the much-needed access to education. However, the private sector is mainly focusing on the quality education. There has been significant growth in the share of education in the private sector during the last few decades. There has been visible increase in the number of schools, as well as the proportion of children enrolled in these schools. Private sector operates schools in all districts of Gilgit-Baltistan.

c. Government Education Sector

The largest education provider in Gilgit- Baltistan is Government Education Department. There are 1008 government schools in Gilgit-Baltistan, where 115176 students get education from 4946 teachers. For the closer and effective monitoring of schools, offices have been established in each district led by the deputy directors of education (DDEs). There are four directors of education to supervise and monitor the DDEs, they are: (i) the director education (academics), Gilgit, the director education, Baltistan, the director education, planning and development and the directress for the girls' schools in Gilgit Baltistan. The directors of education are accountable to the secretary education that provides the overall leadership to the institution.

Problem statement

This study was conducted at a junior high school and senior high school in California. The authors found that absenteeism was a significant problem in the school, but the organization, of the school was not designed to help eliminate the problem. As ubogu (2004) identified that illness, permitted leave, voluntary absenteeism, as common forms of absenteeism. The first factor discussed division of labor, pointed out the tendency of the school personnel to pass the responsibility for attendance policy enforcement back and forth among them, rather than taking direct responsibility for it. Another organizational, concern involved the classification of absenteeism as a problem. Most teachers and administrators resisted this classification because it involved direct enforcement of a compulsory attendance regulation. Teachers and administrators did not want to nay the professional or psychic price to do so the system of rewards and sanctions for teachers who did or did not enforce. Attendance policies were not very strong. Wright (1978) found statistically significant differences by location: urban schools had the lowest attendance rates, then suburban schools. The students also did not have a very strong system of rewards or sanctions presented to them. The authors also found major deficiencies in the schools policy making and training procedures.

Absenteeism not only disturbs the academic development of the student, but also effects the in-class planning of teachers [6]. Deprived economic situations of families also play a noteworthy role in absenteeism [5].Many of the researchers reported that absenteeism is everywhere in their country [1] [2] [9] [10].

It is a general opinion of the elders that students do not attend their schools regularly and this situation is worst in public sector schools. As the researcher is a teacher in public sector and student of education, this phenomenon is directly related to his job and degree of M.A. Education. Researcher took

this phenomenon as his research study to explore intensity, root causes and possible measures to overcome this problem.

OBJECTIVES OF STUDY

1. To find out the perceptions of teachers regarding causes of absenteeism of student at Secondary School Certificate (SSC) level in Baltistan.
2. To investigate the role of teacher in minimizing Absenteeism of their respective Students.
3. To find out the contribution of Head teacher for minimizing Absenteeism of Students at Secondary School Certificate Level.
4. To find out the cooperation and contribution from parents in minimizing Absenteeism of Students at Secondary School Certificate Level.
5. To give recommendation to reduce Absenteeism of students in Secondary School Certificate Level education in Baltistan.

SIGNIFICANCE OF THE STUDY

This research study might be helpful for:

- Teachers, to locate the problems that are cause of absenteeism.
- Managers, to modify/ make necessary changes to ongoing practices.
- Parents, to play their role to minimize/ eradicate absenteeism of their children.
- Students, to get benefits from the findings and recommendations of the study.
- Curriculum Developers, to include more interesting activities as according to psychological needs of the students.

METHODOLOGY

Any research work can be fruitful only if conducted by proper method in a systematic manner. The investigator has adopted mix method that is qualitative and quantitative.

POPULATION OF THE STUDY

Teachers of all 38 male and female secondary schools in public sector of district Skardu included in population of the study.

Table 1. Number of Schools in District Skardu

S.No	Tehsil	Boys	Girls	Total
1	Skardu	5	5	10
2	Gambaskardu	5	2	7
3	Roundu	4	1	5
4	Gultari	1	0	1
5	Kharmang	4	3	7
6	Shigar	5	3	8
Total		**24**	**14**	**38**

SAMPLE OF THE STUDY

Researcher used Stratified sampling technique to select the sample of the study from given population. Stratified sampling will facilitate the researcher to identify and get data from sub groups in the population (Tehsils) as according to their proportion.

There are six Tehsils in Skardu District. (Skardu, Gamba Skardu, Roundu, Shigar, Gultari and Kharmang) Researcher divided these six Tehsils in to two groups that is A and B. Group A compresses Tehsil Skardu, Tehsil Gamba Skardu and Tehsil Roundu while Group B compresses of Tehsil Shigar, Tehsil Gultari and Tehsil Kharmang. Sample is taken as 40% of total available schools. Detail of sample school is given below in Table.

Table 2. Sample of the study

Group A				
S.No	**Tehsil**	**Boys**	**Girls**	**Total**
1	Skardu	2	2	4
2	Gamba Skardu	1	1	2
3	Roundu	1	1	2
Group B				
1	Gultari	1	0	1
2	Kharmang	2	1	3
3	Shigar	2	1	3

For the purpose of data collection Researcher contacted senior most Three (3) teachers of institutions as given/ nominated by school administration.

DATA COLLECTION

Researcher used only single tool for his study. Specific questionnaire for teachers of public schools are constructed to get their feedback regarding causes of absenteeism in students of Baltistan. For the purpose of data collection, researcher developed questionnaires for teachers utilizing three points rating scale, open ended and closes ended statement. The data was collected by researcher himself and as well as by the help of research assistant.

DELIMITATION

Keeping in view limited time and financial constraints, this study was delimited to all public sector secondary schools (Male/Female) in District Skardu.

ANALYSIS

Table 3. Frequency of each item

S. No	**Statement**	**Strongly Agree (%)**	**Agree (%)**	**Disagree (%)**
1	Teacher's behavior is the cause of absenteeism of students from school.	23.3	50.0	26.7
2	Absenteeism of students from school is due to the domestic activities.	23.3	60.0	16.7
3	Reason of absenteeism of students is the long distance of school.	16.7	26.7	56.7
4	Lack of facilities at school is the cause of absenteeism of students from school.	30.0	40.0	30.0
5	You think that workload at school keep students away from classes.	13.3	36.7	50.0
6	Physical health of students affect their punctuality.	33.3	56.7	10.0
7	Cause of absenteeism of students may be the Head teacher's behavior.	3.3	26.7	70.0
8	Students cannot attain the classes regularly due to peer behavior.	16.7	50.0	33.3
9	Lack of awareness of education by parents is the cause of irregularity of students.	63.3	26.7	10.0
10	Absenteeism is due to the combinations of subjects opted is not as according to students aptitude.	13.3	33.3	53.3
11	Excess of luxuries from family keep students away from school.	26.7	46.7	26.7
12	Community participation can reduce student's absenteeism.	56.7	40.0	3.3
13	You regularly inform parents about student's performance.	33.3	40.0	26.7
14	You provide incentives to punctual students.	30.0	56.7	13.3
15	You inform head teacher about student's performance?	53.3	43.3	3.3
16	In staff formal meeting you highlight absenteeism of students as serious issue.	43.3	53.3	3.3
17	You provide guiding and counseling session for students who remain absent.	30.0	40.0	30.0
18	Performance of students can affect by absenteeism.	50.0	40.0	10.0
19	You are providing additional lectures for absent students.	13.3	43.3	43.3
20	Punishment can reduce student's absenteeism.	30.0	63.3	6.7

Table 4. Suggestions from Teachers to reduce Student's Absenteeism

S. No	Responses	f	%	Ranking
1	Give awareness to the Parents about education.	26	86.7%	1
2	Inform Parents about their kid's performance on regular bases.	23	77.7%	2
3	Provide guiding and counseling session.	22	73.3%	3
4	Avoid punishment	18	60.0%	4
5	Make classroom environment friendly for students.	17	56.7%	5
6	Involve community in school matters.	15	50.0%	6

RESULTS AND DISCUSSION

Table 3 of analysis showed that majority of the teachers strongly agreed or agreed while the remaining few teachers disagree by the statement. The cumulative percentage of strongly agreed and agreed is 73.3% so it is proved that teacher's behavior affected the regularity of the students. Next the result of the statement that work load at school is cause of absenteeism. Different sample with different frequency that is Half sample strongly agreed and agreed only while other half sample disagreed by the statement .Here work load at school means the homework, Class work and patron of examination etc. 50% favor and 50 % oppose the statement .So it is very difficult to decide that it may a cause or not a cause of absenteeism. Next when researcher asked to the study samples that "is the physical health of students' cause of absenteeism? Collectively majority of the sample agreed the statement. So it clearly describes that the statement is very true and one of the major cause of absenteeism of students from their classes at secondary level. Maximum sample disagreed the statement which showing the result that head teachers behavior is not the cause of absenteeism of students for school. It is comes to know that Lack of awareness of education by parents may be a cause of absenteeism of students from school because majority of the teachers strongly agreed, while only few teachers disagreed by the statement. The cumulative percentage on positive response is 90 %.

Combination of opted subject is not according students aptitude is the cause of students' absenteeism because maximum teachers are in favor of this statement. This result shows that the subject opted by the parents or teachers or students which is not according to their interest is a serious issues which reduce the interest of students about studies. Similarly, majority of the sample was in the favor of the statement that excess of luxuries from family is the cause of absenteeism of students from school. The cumulative percentage of agreed sample because 73.3 %. It means that when the students get excess of luxuries during school life its put negative impact on their studies so we can easily describe that excess of luxuries by family is one of the key cause of student's absenteeism.

Majority of the teachers are in the favor of the statement that reward can reduce student absenteeism. According to table cumulative person in favor of the statement is 93.3%. It is very easy to say for the researcher that reward can reduce student absenteeism from school at secondary level. On the other hand we can say that school environment and teacher's behavior is directly impact on student's performance. Reward increases the interest of student towards study. Punishment cannot reduce student absenteeism. Here majority of the studied sample disagreed by giving their arguments in oppose of the statement. It comes to know during data collection and by data analyze that punishment can't reduce students absenteeism from school. But few teachers were in the favor of the statement that at some time and some contacts punishment can be help full to make students regular for their studies. Finally, majority of sample said that maximum teachers are in favor of punishment by fine. So it is described that majority of the teachers punished there students by giving financial penalty when they become absent from school.

CONCLUSION

Teacher's behavior is the causes of absenteeism of students from school at secondary level. Because Baltistan is one of the remotest areas of Pakistan, there is lack of facilities for students in public sector schools. The physical health of students is also a cause of absenteeism at secondary level. Maximum teachers thought that reward can reduce student's absenteeism while few of them are in the favor of punishment to reduce student's absenteeism. They think that punishment by giving them a financial penalty can help to reduce student's absenteeism from school.

RECOMMENDATIONS

One to base of conclusions the following some recommendation and suggestion are writing down to reduce students absenteeism from school.

- Give awareness to parents about education.
- Teachers should provide healthy and friendly environment for students.
- The parents should also manage time to see whether their children's completed the home task assigned to them by their teachers.
- All the social agencies and the members of the society or community should release the importance of education for their children.
- Due to long distance from schools students remain absent. This factor should be resolve by providing them better transport facilities and setting up of boardinghouses for those students who are coming from far area.

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Endophytic Fungi Isolated from *Citrullus Colocynthes*l. Leaves and Their Potential for Secretion of Indole Acetic Acid and Gibberellin

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ABSTRACT

Fungal endophytes are the best known beneficial partners of all plants, having role in providing immunity against biotic and abiotic stresses as well as synthesizing biologically active secondary metabolites including plant growth promoting hormones. Twenty-nine (29) different fungal strains were isolated from xerophyte plant *Citrulluscolocynthes* L. (colocynth/bitter apple) leaves and screened on rice seedlings for their growth promoting or inhibiting activities. 20 strains were found to be growth promoter, 2 growth inhibitor and 7growth neutral. Growth promoting potential of endophytic fungi was further confirmed by detecting and quantifying gibberellic acid (GA_3) and indole acetic acid (IAA) in their culture filtrates via PerkinElmer Lambda 25 double beam spectrophotometer. Highest GA_3 concentration was found in the culture filtrate of CL 1-5-11 (146.06ng/ml) while, least in CL 1-2-1B (1.41ng/ml). Similarly, highest concentration of IAA was detected in the culture filtrate of CL 1-2-7 (50.66µg/ml) while, least in CL 1-2-6A (3.5µg/ml). Present work aims to replace artificial fertilizers by bio-fertilizersas they are cheap and non-hazardous to environment and humans as compared to artificial fertilizers.
KEY WORDS: Endophytic fungi, Gibberellin, Auxin, *Citrulluscolocynthes* L.

INTRODUCTION

Man is in continuous struggle to fulfill the need of food for over-increasing population of the world. For the better quality and yield of crops, chemical fertilizers are used excessively. But these chemical fertilizers are hazardous to human and environment. Thus, use of bio-fertilizers in the agricultural world in the form of microbes like fungi, bacteria and cyanobacteria, may be one of the most suitable alternates and safe options [1]. Endophytes are mutualistic symbionts of all plants found completely inside them and cause no harm to host plant. High density of endophytic fungi has been recorded in the temperate and tropical rain forests[2]. Endophytic fungi are a diverse group and commonly belong to Ascomycota which reside in plant roots, stem, leaves, flowers, fruits and spines [3]. Endophytic fungi have positive role for host plants regarding phytohormones synthesis, N_2 fixation, P absorption and Phosphate solubilization [4]. They also release toxins to make plants unfit for herbivores feeding as well as enhance production of phytohormones (Cytokinin, Gibberellin and Auxin) that widens host potential for limited resources [5]. They have beneficial role for enhanced ecological adaptability of host plant and more biomass production [6]. It has been studied that *Phomaglomerata*and *Penicillium* sp., promote Waito-C and Dongjin-beyo rice (GAs deficient dwarf mutant) growth under salinity and other stresses as a result of biologically active secondary metabolites secretion (IAA and GA_1, GA_3, GA_4 and GA_7) as well as reduce ABA synthesis. Other stress related activities of plants like catalase, per-oxidase, polyphenol oxidase, ascorbic acid oxidase and glutathione also decreases under different stresses in fungal-endophyte-colonized-plants [7]. Phytohormones are secondary metabolites that function as signaling compounds and having role in important physiological activities of plants (growth and development). IAA and GAs are the two important plant hormones, but currently little is known about their secretion by endophytic fungi [8]. Current study aims to know the GAs and IAA secreting potential of endophytic fungi, a promo to replace costly and hazardous chemical fertilizers by safe, cheap and eco-friendly bio-fertilizers.

MATERIALS AND METHODS

Isolation of endophytic fungi

Leaves of *Citrulluscolocynthes* L. were collected from District Nowshera of Khyber Pakhtunkhwa, Pakistan, carried to plant-microbes-interaction (PMI) laboratoryand washed with water. Samples were then centrifuged inTween 80 solution to remove air born dust particles, at 120 rpm (revolutions per minute (rpm) for five (5) minutes at room temperature, in shaking incubator [9]. Then surface sterilization was done using ethanol (70%) for half minute, NaOCl (Sodium Hypo Chloride) (3%) for 5 minutes, and again (70%) ethanol for half minute. Leaves were cut into

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small discs (3mm) with- and without-midribs with the help of cork-borer [10]. Leaf pieces were then placed on Hagem minimal medium containing 80ppm streptomycin. Plates were kept at room temperature for 5 days [11]. After the emergence, endophytic fungi were inoculated on PDA (potato dextrose agar) medium for purification. For IAA and GAs collection, Czapek broth medium was used [12, 13].

Growth medium and culture conditions

Hagem, a minimal medium ($C_6H_{12}O_6$ 5gm/L, KH_2PO_4 0.5gm/L, $MgSO_4.7H_2O$ 0.5gm/L, NH_4Cl 0.5gm/L, $FeCl_3$ 0.1gm/L, Agar 15gm/L and Streptomycin 0.08gm/L (pH 5.6 ± 0.2), was used for the isolation of fungal strains from the leaves of *Citrulluscolocynthes* L. PDAmedia (Dextrose 20gm/L, agar powder 20gm/L, 300gm potato (sliced washed unpeeled) and 1L DW) was used for the pure culture of endophytic fungi in plates as well as slants. Slants were kept in refrigerator at 4ºC for storage. Then 50 ml Czapek media($C_6H_{12}O_6$ 10gm/L, peptone 10gm/L, KCl 0.5gm/L, $MgSO_4.7H_2O$ 0.5gm/L, $FeSO_4.7H_2O$ 0.5gm/L, pH 7.3 ± 0.2) was used in 250 ml flasks for the collection of active secondary metabolites (GA_3 and IAA). Flasks were kept at 30ºC in shaking incubator (120 revolution per minute) for 7 days [12, 13].

Screening bioassay

On the basis of colonial morphology and growth, different types of fungal strains were selected for further analysis. Culture filtrates of twenty-nine (29) strains out of 98, were screened on rice plants for their growth promoting potential. One ml supernatant obtained by growing endophytic fungi in Czapek broth media, was diluted in autoclaved DW and applied on the tip of rice plants at two leaves stage. Rice seedlings were grown in glass beakers having 30 ml media (0.8% water-agar medium) [12, 13]. With the help of micro-pipette, 100µl of fungal extracts were applied on the tips of experimental while, DW and Czapek medium on control rice seedlings. Rice seedlings were shifted to growth chamber (day/night cycle: 14 h—28 °C ± 0.3; 10 h—25 °C ± 0.3; relative humidity 70%;) six (6) plants per treatment. Root and shoots length, fresh and dry weight of rice plants were recorded after 7 days of the application of fungal extract [11].

Determination of gibberellin and auxin

A very simple and accurate bioassy of Ismail *et al*. [1] with some modifications, was used for the detection and quantification of GA_3 in the culture filtrate of endophytic fungi while, Salkowski reagent was used for the determination of IAA [14]. Salkowski reagent and fungal filtrate were mixed in 2:1 ratio and incubated for 30 minutes in the dark at 25^0C. IAA was determined with PerkinElmer Lambda 25 spectrophotometer at 540 nm wavelength.

Standard curve for GA_3

Five different concentrations of pure GA_3 were made and their OD (optical density) were checked at 254nm with spectrophotometer (PerkinElmer Lambda 25).Standard curve of GA_3 was linear up to 5 order of magnitude with R^2 = 0.999998 (Figure 1a).

Standard curve for IAA

Ten different concentrations (starting from 10 µg/ml to 100 µg/ml) of IAA were made and OD was taken at 540nm with spectrophotometer (R^2 = 0.970196, Figure 1b).

Data analysis

Duncan Multiple Range (DMRT) was applied for data analysis. (IBM SPSS software version 21.0, SPSS Inc, Chicago, USA).

RESULTS AND DISCUSSION

Endophytic fungi isolated from *Citrulluscolocynthes* L.

On the basis of morphological differences, twenty-nineendophytic fungi were isolated from *Citrulluscolocynthes* L. leaves (Figure S1). The fungal culture filtrates of these endophytes were bio-assayed on rice plants for growth promoting or growth inhibiting activities (Figure S2). 29 fungal filtrates were applied, of which 20 were found as growth promoter, 2 as growth inhibitors while, 7 as growth neutrals (Table 1).

Table 1: Screening bioassay of fungal culture filtrates on rice seedlings isolated from*Citrulluscolocynthes* L.

Fungal isolates	Shoot length (cm)	Root length (cm)	Fresh weight shoot (g)	Fresh weight Root (g)	Dry weight shoot (g)	Dry weight Root (g)	Total chlorophyll (SPAD)	Growth status
Control DW	7.7±0.3	4.9±1.	.03±0.0	.088±0.03	.005±0.01	.015±0.01	14.3±3.4	
Control Czk	8.6±0.4	4.2±2.	.034±0.0	.093±0.01	.007±0.03	.014±0.01	15.7±2.3	
CL 1-1-1A	13.9±0.	5.4±1.	.067±0.0	.099±0.09	.01±0.01	.026±0.01	19±2.1	Promoted
CL 1-6-4	13.1±1.	6.1±1.	.071±0.0	.099±0.02	.009±0.02	.026±0.01	18.9±1.7	Promoted
CL 1-3-5	11.7±1.	4.5±2.	.046±0.	.061±0.08	.005±0.03	.015±0.02	15.9±1.2	Promoted
CL 1-5-11	13.4±0.	7.6±0.	.084±0.0	.088±0.03	.01±0.04	.031±0.01	18.4±1.8	Promoted
CL 1-5-10	14±0.	5.2±1.	.096±0.0	.097±0.07	.013±0.04	.029±0.01	19.7±3.2	Promoted
CL 1-6-3	11.2±1.	6.5±2.	.046±0.0	.061±0.04	.005±0.01	.015±0.01	15.9±2.6	Promoted
CL 1-6-6	5.5±1.	2.7±1.	.013±0.0	.04±0.06	.0001±0.03	.005±0.02	11±4.1	Inhibited
CL 1-2-2	7.5±0.	3.5±2.	.036±0.0	.056±0.05	.003±0.04	.009±0.03	13±2.9	Neutral
CL-1-1-3A	12.2±0.	7.2±3.	.083±0.0	.088±0.06	.01±0.02	.031±0.01	18.4±2.1	Promoted
CL 1-2-6B	12.7±1.	6±1.	.082±0.0	.087±0.05	.009±0.02	.033±0.01	18.5±5.3	Promoted
CL 1-6-2	9.2±0.	3.5±0.	.034±0.0	.026±0.07	.008±0.01	.004±0.02	13.7±3.2	Neutral
CL 1-2-4	13±0.	4.4±0.	.08±0.0	.089±0.03	.009±0.04	.031±0.01	18.4±2.7	Promoted
CL 1-4-2	14.1±1.	6.6±1.	.096±0.0	.097±0.08	.013±0.03	.029±0.02	19.7±3.4	Promoted
CL 1-1-3B	13.2±0.	5.8±1.	.083±0.0	.083±0.03	.01±0.04	.032±0.02	18.7±4.2	Promoted
CL 1-2-8	13.6±1.	5.9±0.	.084±0.0	.082±0.09	.008±0.01	.034±0.01	18±2.6	Promoted
CL 1-1-1B	11.4±0.	4.5±1.	.047±0.0	.061±0.02	.005±0.01	.015±0.01	15.9±3.2	Promoted
CL 1-5-2	11.5±0.	4.7±0.	.045±0.0	.06±0.01	.006±0.03	.013±0.01	15.2±1.5	Promoted
CL 1-1-4	7.5±0.	6±1.	.013±0.0	.04±0.09	.0001±0.02	.005±0.02	11±1.3	Neutral
CL 1-2-1B	13.5±1.	6.7±0.	.081±0.0	.088±0.02	.009±0.02	.031±0.02	18.4±1.8	Promoted
CL 1-2-3	10.5±1.	5±0.	.046±0.0	.061±0.08	.004±0.04	.015±0.01	15.9±4.3	Neutral
CL 1-2-7	13.5±0.	7.3±1.	.084±0.0	.083±0.03	.009±0.05	.031±0.01	18.4±2.7	Promoted
CL 1-2-6A	12.2±1.	5.5±1.	.057±0.0	.065±0.07	.006±0.01	.013±0.01	15.9±1.8	Promoted
CL 1-6-14	10.2±0.	6±0.	.047±0.0	.061±0.04	.004±0.01	.015±0.03	15.9±3.2	Neutral
CL 1-3-3	4.4±1.	5±1.	.013±0.0	.041±0.06	.0001±0.02	.004±0.01	11±1.6	Inhibited
CL 1-2-1A	11.5±0.	4.5±0.	.045±0.0	.065±0.05	.004±0.01	.013±0.01	15.9±2.5	Promoted
CL 1-2-9	14.5±1.	7.5±1.	.095±0.0	.094±0.04	.014±0.05	.029±0.02	19.7±3.4	Promoted
CL 1-3-1	9.8±1.	4.5±0.	.047±0.0	.06±0.06	.004±0.01	.013±0.02	15.9±1.3	Neutral
CL 1-1-2	11.4±0.	5.5±1.	.045±0.0	.063±0.03	.004±0.03	.015±0.01	15.9±2.6	Promoted
CL 1-6-11	10.7±1.	4.9±1.	.041±0.0	.06±0.07	.004±0.01	.013±0.01	15.9±1.7	Neutral

Note: Czk= Czapek Medium, D.W = Distilled Water. Values with different letters in the same column in that group are significantly different at the 5% level by DMRT (Duncan's Multiple Range Test). Values within the table refers to the mean ± SE (n = 3)

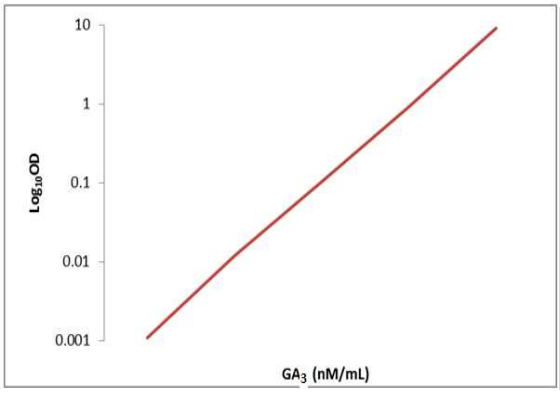

Figure. 1(a). Gibberellic acid (GA_3) standard curve

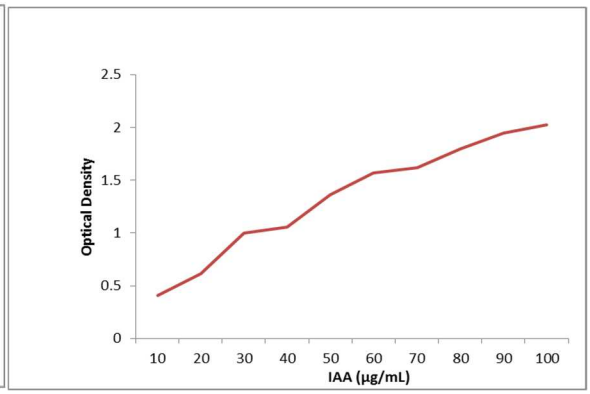

Figure. 1(b). Indole acetic acid (IAA) standard curve

Gibberellic acid determination and quantification

GA_3 detected and quantified using a very simple protocol, used by Ismail *et al*. This is a very simple but accurate bio-assay for GA_3. In this procedure embryo was totally discarded from the seeds of wheat. This bio-assay for GA_3 determination in fungal culture filtrate was first applied by Ismail *et al*. [1]. Highest GA_3 (146.06ng/ml) concentration was shown by strain CL 1-5-11 while, least (1.41ng/ml) by CL 1-2-1B (Figure 2).

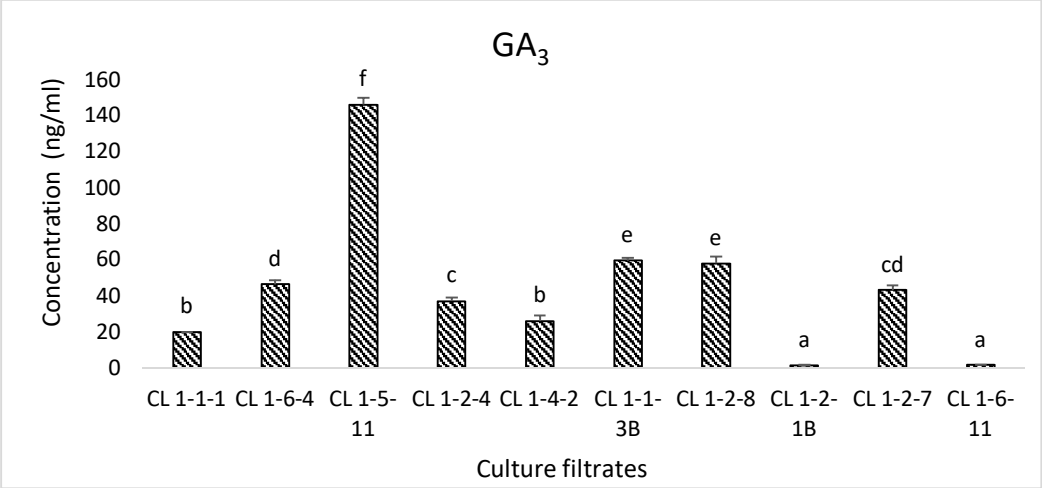

Figure 2: Quantification of GA_3 in fungal extracts. CL 1-5-11 have highest (146.06ng/ml) GA_3 concentration while, CL 1-2-1B have least (1.41ng/ml)

Indole acetic acid determination and quantification

IAA was determined in culture filtrates using Salkowski reagent. Salkowski solution gives a pink color in the presence of IAA which can be easily detected in the filtrates. Total of 29 fungal extracts were checked, 11 were found to have IAA. Two strains, CL 1-2-7 and CL 1-1-1 were maximum IAA producer 50.66µg/ml and 48.18µg/ml respectively while, CL 1-2-6A was the least producer strain (3.5µg/ml) (Figure 3).

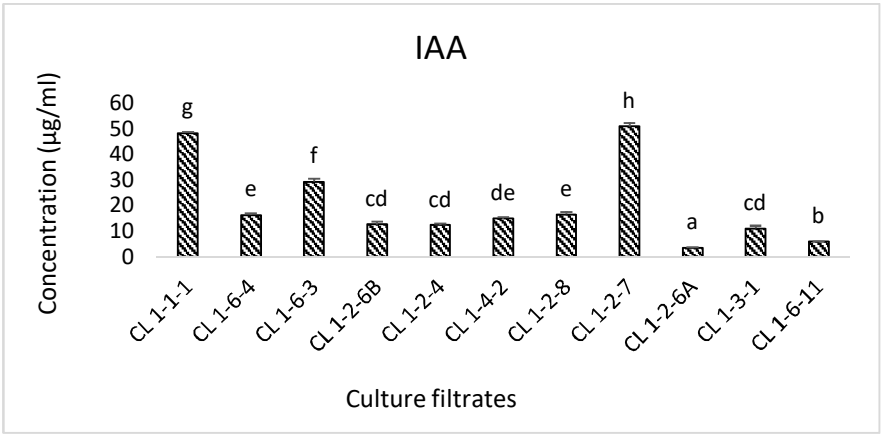

Figure 3: Quantification of IAAin fungal culture filtrates. Highest IAA (50.66µg/ml) concentration was found in strain CL 1-2-7 while, least (3.5µg/ml) in CL 1-2-6A strain

More than 0.3 million herbaceous and woody land plant species have been checked so for, for the presence of endophytic fungi. All of these plants are known to colonize one or more endophytic fungi [3]. All endophytes are known to have mutualistic relationship with their colonized host plant [15]. Endophytic fungi not only provide immunity to host plants against different stresses like pathogenicity, herbivory, drought, high and low temperatures and salinity but also having role in nitrogen fixation, phosphate solubilization, phosphorus absorption and phytohormones production [4]. In this work fungal filtrates were screened on rice seedlings for the presence of GA_3 and IAA as both hormones have potential role for plant growth promotion. Presence of GA_3 and IAA was also confirmed via PerkinElmer Lambda 25 spectrophotometer [16, 17]. Therefore, culture filtrate of endophytic fungi is considered to be the best source of biologically active secondary metabolites[14]. Screening bio-assay of fungal extracts on plants, is one of the easy and simple procedure for the determination and active secondary metabolites [18, 19]. Rim *et al.* [20] also used the same procedure for the screening of *Fusariumproliferatum* filtrate. Highest GA_3 (146.06ng/ml)concentration was shown by strain CL 1-5-11 while, least (1.41ng/ml) by CL 1-2-1B as compared to just 3.21ng/ml detected by [12]. Similarly, highest IAA concentration (50.66µg/ml), was detected in CL 1-2-7 strain while least (3.5µg/ml) in CL 1-2-6A strain.

CONCLUSION

Present work indicates that fungal endophytes form mutualistic relationship with plant species. Presence of 29 endophytic fungi in one xerophytic plant suggests their diversity and adaptation to different habitats. Further, detection of biologically active secondary metabolites (GA_3 and IAA) in their culture filtrates, show their important ecological role for supporting host plants. Use of endophytic fungi in the agricultural field as bio-fertilizer, is suggested for the future because of hazardous effects of artificial fertilizers on the environment and human beings.

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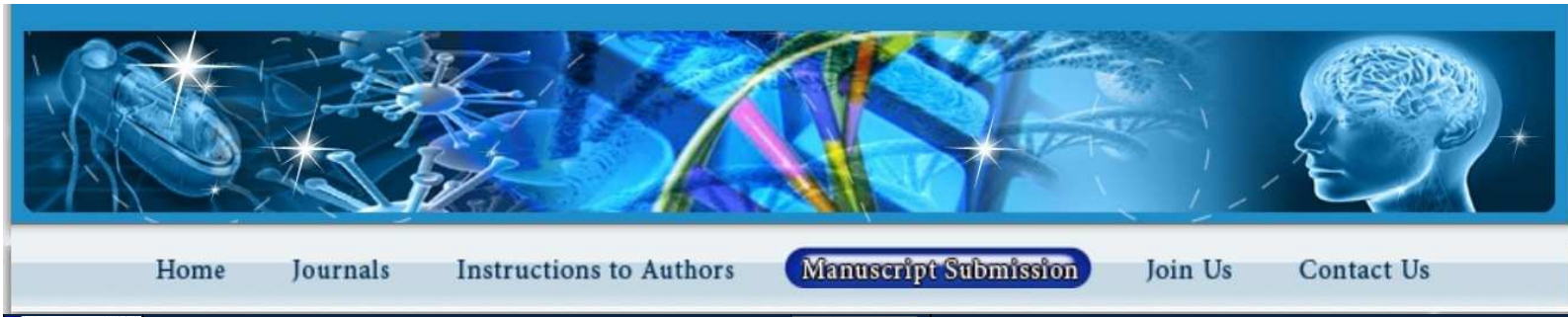

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Books:

1. Daniel A. Potter, 2002. Destructive turfgrass insects: Biology, diagnosis and control. Wiley Canada Publishers, pp: 24-67.

Chapters in Book:

1. Bray R.A., 1994. The leucaena psyllid. In: Forage Tree Legumes in Tropical Agriculture (eds R.C. Gutteridge and H.M. Shelton) pp. 283–291. CAB International, Oxford.

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A Report:

1. Makarewicz, J.C., T. Lewis and P. Bertram, 1995. Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan, 1983-1992. U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference Proceedings:

1. Stock, A., 2004. Signal Transduction in Bacteria. In the Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.

A Thesis:

1. Strunk, J.L., 1991. The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior, M. S. thesis, Michigan State Univ., East Lansing, MI.

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