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Scope

Journal of Applied Environmental and Biological Sciences (JAEBS) is a peer reviewed, open access international scientific journal dedicated for rapid publication of high quality original research articles as well as review articles in the all areas of Applied Environmental and Biological Sciences.

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J. Appl. Environ. Biol. Sci. 2018 8(6): 77-86. [Abstract] [Full-Text PDF] [Full-Text XML]

A Review on Climate Change, Vulnerability to Major Crops and Biotechnological Adaptations in Pakistan

**Hajra Ahsan[*], Sumbal Zafar, Maham Ayesha, Madiha Alam, Sidra Alam,
Sabahat Farooq, Sana Iqbal, Nayab Komal**

Department of Environmental Sciences, University of Gujrat, Gujrat, Pakistan

ABSTRACT

In the drivers of globalization, climate change is a major concern on global level but not only on globe scale but in Pakistan also. Climate change and its tremendous climatic conditions are threat to major crops of Pakistan. So, Pakistan is one of the vulnerable countries to climate change. In this study, we will examine the major crops like wheat, rice, cotton, sugarcane and maize and how these crops effecting by climate change and its harsh conditions including maximum and minimum temperature, rainfall pattern/precipitation, and relative humidity and day night effects. Studies reveal that maximum temperature is harmful for wheat production but optimal temperature is positively important for all crops (cotton, sugarcane, maize and rice). Precipitation has negative effects on all crops except rice. To overcome the effect of climate change modern technologies of biotechnology can be used that are drought resistant including genetically modified plants that can grow under stress, energy-efficient and transgenic plants of high yield. Good biotechnology improvement is contributing in increasing the agricultural productivity.
KEYWORDS: Climate change; Pakistan; Production; Major crops; Biotechnological agricultural development

INTRODUCTION

Evidence shows that there is increase on global temperature since 19 century [16]. The first decade in previous 21 century recorded the warmest decade as compare to previous decades. Since 1950 the global temperature has been increased by 0.72% [16]. Due this phenomenon there is decline in number of cold days and nights and increase in hot and dry days and nights. Intergovernmental Panel on Climate Change (IPCC) reveal that change in climate is contributing in the global warming by emitting greenhouse gases include vaporous gases, carbon dioxide which is main contributor of global warming. IPCC also studied that at the end of 21 century temperature increase by 0.3 – 4.8^{0}C [7].Other gases that bringing change in climate include methane and nitrous oxides also changing rainfall pattern, temperature and negative effect on ecological resources e.g. water and land resources. All these factors that contributing the change in climate also casing phenomenon of flooding and drought. The change in temperature causing intensification of greenhouse gases. The consequences of these variations in weather causing glacier melting, increase in rainfall and shifting in seasons as prolonged summer season.

Climate change is a global phenomenon that is not only present on global level but widely increasing in developing countries including Pakistan. The increasing trend of climate change in developing countries is due to more vulnerability and less remediation to overcome the climate change. Developing countries including Pakistan that relay on agricultural sector. During the last 50 years this division is realized as a major industrious sector of Pakistan's economy. 61% population is living in more than 50,000 villages in Pakistan. This sector is at more risk because agriculture is effecting badly due to extreme temperature. Other factors like high precipitation, flooding, drought, degradation of land resources also causing damage to agriculture [22]. As agriculture is more susceptible to change in climate. Due to climate change the production of pests and weeds reducing the requires yield of crops.

Change in precipitation pattern causing the short and long run deterioration of crops. It is predicted that till 2050 about 9 Billion people will face the problem of food uncertainty due to reduction in agricultural yield. The countries at low altitude including arid and Sami-arid areas where availability of water is low are at most risk to climate change effect on crop yield. Studies revealed that about 1-2^{0}C warming temperature is threatening to crops. This is due to decrease in moisture content in soil and increase in evapotranspiration of the plants. According to the IPCC scenario of future emission of CO_2, now the concentration of CO_2 is about 379 ppm but in future it could be reached at 550 ppm in 2100 [39]. Carbon dioxide act as fertilizer to crops and enhance the ability of photosynthesis. So, increase in the concentration of CO_2 is beneficial to crop yield but on other hand it is major contributor in climate

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Corresponding Author: Hajra Ahsan, Department of Environmental Sciences, University of Gujrat, Gujrat, Pakistan.
Email address: hajrahsan15@gmail.com (Hajra Ahsan)

change as greenhouse gas. Extreme events of climate change effecting crop production as increasing phenomenon of droughts and flooding causing damage to agricultural lands which is decreasing the yield of crops and decline economic activities [4]. Thus, to meet the reducing goals of greenhouse gases there is need to require mitigation strategies to overcome the change in climate.

Agriculture in Pakistan

Agriculture sector is the backbone of Pakistan's economy. This sector makes the 26% of gross domestic production (GDP) and supporting a large of population. Major crops of Pakistan including Rice, wheat, maize, sugarcane, cotton, fruits and vegetables that all vegetated by irrigation system which is consider as world's largest irrigation system. There are two seasons for crop production one is from May to November for production of cotton, rice, maize and from November to April for wheat production. This sector involves in the great economic role of export and import and enhancing the irrigation and marketing green revolution system [34]. The annual export of Rice is about 2 million tons worldwide. Rice is fulfilling the 60% of population food needs [8-19]. Pakistan share 9.8% cotton worldwide and largest cotton producer ranked as 4th in the world in 2011 - 2012. It provides employment to about 35% of population in Pakistan [12-15]. Maize is also cash crop of Pakistan. Sugar cane contributing in 0.7% of GDP of Pakistan and it is using as biofuel in the world [35].
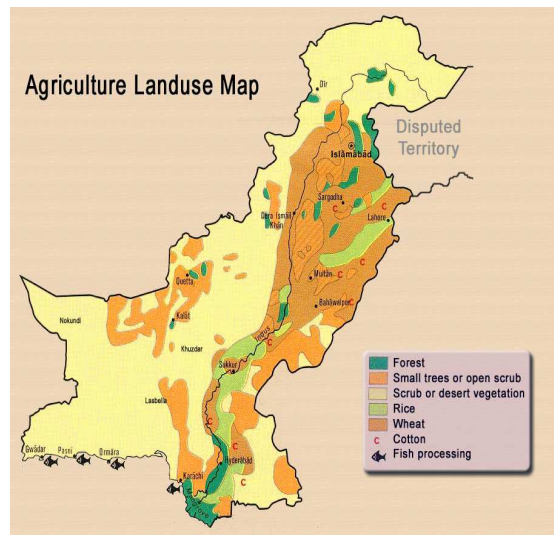
Source: Pakistan Metrological Department

Figure 1: Land use and agriculture map of Pakistan

All crops depend on the water for growth but the availability of water effect the yield of crops which is predicted to decrease about from 1995 to 2020 in the range from 72-62% in developing countries [20]. There is great challenge for agriculturalist to produce sufficient crop for the growing population in Pakistan [12]. Therefore, it is suggested that there is need for the new and developed agricultural funding programmes in Pakistan.

Climate change in Pakistan

In most of the climate change susceptible countries Pakistan is ranked eight according to German Watch. In 2008 Pakistan's total emission of GHG were 310 million tons equivalent to carbon dioxide. According to National GHG inventory 2008 the amount of greenhouse gases is Carbon dioxide 54%, methane 36%, nitrous oxide 9%, Carbon Monoxide 0.7% and VOCS 0.3%. climate change effecting the volume of rivers of Pakistan by melting of glaciers. Studies investigate that the climate of Pakistan is getting harsh due to ongoing increase in temperature and temperature.

A global emission scenario predicted that Pakistan's temperature is rise by $3 - 5^0C$ at the end of this century. IPCC predicted that is would be higher rate of increasing temperature than global level that will impact the glaciers and monsoon rainfall pattern. As a result, this will damage the yield of agricultural crops and energy sector which relay on water availability.
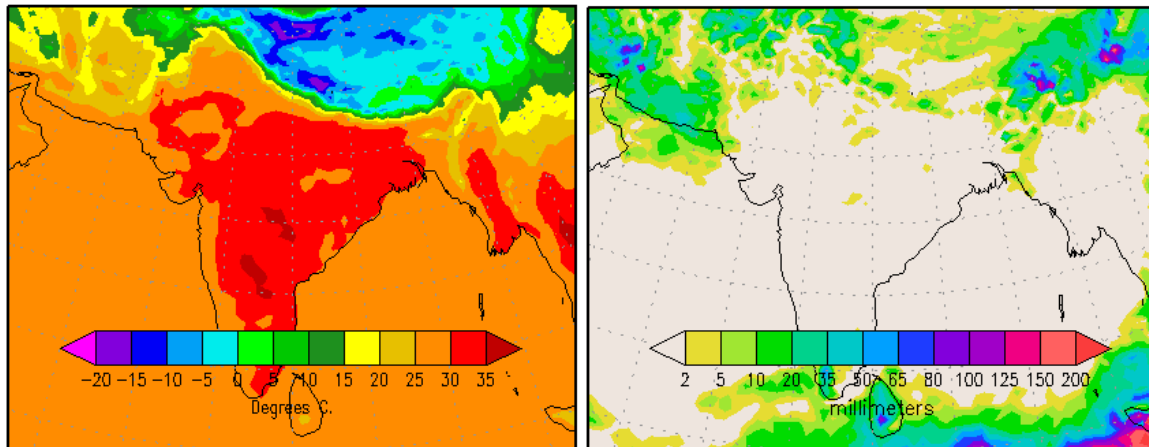
Source: Pakistan Metrological Department

Figure 2: Predicted precipitation (mm)   *Figure 3: Average temperature (^0C)*

Figure 2 and 3 shows the predicted outlook of change in temperature and precipitation from April 7 to April 10 in 2013 according to Pakistan weather porta

Crop yield response to Climate change
To study climate change impact on agriculture sector in Pakistan it is also important to study the sensitivity of crops in harsh temperature. There is increase in CO_2 concentration due to climate change that may positively effect the crops and increasing temperature and precipitation effect the biological activities to work efficiently in growth of crops. We observed 30% production loss due to climate change. The antagonistic effects of climate change on the agricultural sector of Pakistan could cause devastation in the country. Due to loss of 2.6 million acres of land resources and increasing flooding given rise to increase in food prices. Most of the industries are effecting due to climate change because they are dependent on agriculture.
Rabi and kharif are two crop seasons in Pakistan. Rabi grown in November to April month and kharif crops grown in May to October. These two seasons make Pakistan an agricultural country and its performance depends on the climate during the whole year. Climate changes generally affect agriculture through changes in temperature, precipitation. Since 2010, the agricultural sector is at more risk in Pakistan due to facing three major flood events in 2010, 2011 and 2014that effect the economy of country. These major events destroy the main crops including vegetables and fruits.

Wheat
Wheat is imparting 9.9% of the agricultural value and 2% in GDP of Pakistan. Winter plants require minimum temperature of 5 to 10^0C. Wheat is also a winter crop that need cold temperature for the dormancy period before flowering season. But the higher temperature increases the weed, disease and pests attack on plants that could effect the vernalization process in wheat. Wheat is the first most crop that effect by climate change because it need coolness and moisture to grow. But this changing weather causing prolonged summers shorter winter seasons. In some places monsoon rainfall pattern also decreasing. Many studies show that wheat is at more risk that other crops to climate change because it needs optimum temperature as compare to other crops: maize, rice, sugarcane and cotton [28].
Wheat yields in winters were likely to decrease by about 14% by 2080. A researcher concluded that wheat yields about 0.3% reduces by 1% increase in temperature. It was resolved that drought considerably reduces wheat productivity. Wheat is amongst C3 crops category. Photosynthesis process suppresses transpiration in C3 crops due to increase in concentration of CO_2. These factors cause to accelerate the growth of wheat plant. However, the CO_2 beneficial effect is offset by increase in temperature. Precipitation also increases the wheat production. From all perspective, it is concluded that climate change is most damaging to wheat because it is temperature sensitive crop. If Pakistan do not adapt the changes in agricultural practices they will lose the productivity of wheat at the end of 21 century. For example, every year in Pakistan floods damage the wheat and other crop production on large number of hectors.
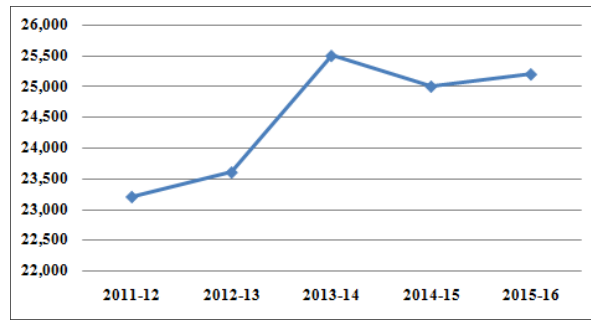
Source: Pakistan Bureau statistics (PBS)

Figure 4: production of wheat (000 Tonnes) in Pakistan

Rice

Rice is second staple crop after wheat and has an important share in export. Rice is grown on different soil characteristics because of its various geographical distributions in worldwide. At the end of this century rice crop is expected to encounter 15-20% reduction due to climate change which is twice of wheat production. Temperature rise was found important for rise production at first when temperature increases beyond certain level the production became destructive toward its production. Study shows that temperature increases by 1.5 - 3.0^0C will enhance the production but increase in more temperature will hurt the rice production.

There was a continuous decline in the yield of Basmati rice in the semi-arid plains of Pakistan increase in temperature up to 5°C.There was about 6% decline in the simulated yield by 1^0C increase in temperature; 12% decrease in grain yield by 2^0C increase in temperature but beyond that the decrease was higher. It was studied that extreme climatic events like heat stress, salt stress, droughts and flooding may damage the rice production [27]. It is drought sensitive so in rain fed system yield may reduce due to climate change factor. It was projected that against temperature studies shows that increase in 1°C temperature the production of rice increases by 16% but reduce yield at 4°C. Carbon dioxide play vital role in photosynthesis. Hence for plant growth increases in carbon dioxide concentration would increases the rate of plant growth and productivity. Due to fertilization effect on crops with C3 photosynthetic pathway, like rice and wheat.
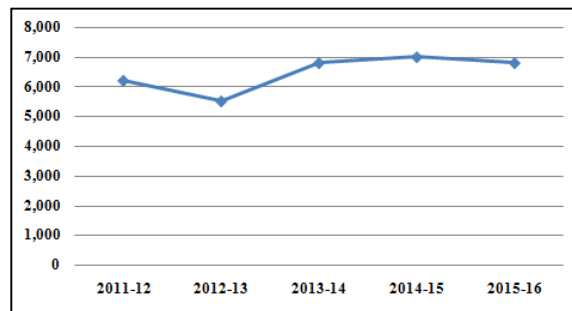
Source: Pakistan Bureau statistics (PBS)

Figure 5: production of Rice (000 Tonnes) in Pakistan

Cotton

Cotton is considering as cash crop for the rural areas of Pakistan because it is source of livelihood for people. It is playing energetic role in the economy of Pakistan [31]. Cotton is grown in hot areas of Pakistan. It is grown in humid and hot areas because it is pest hazardous that suppress the quality of its yield. Major effect of climate change on cotton production due to greenhouse gases emission and leaching effect due to climate change [18]. As the leaching factor effecting the most water bodies of Pakistan by nitrates coming from runoff that is using in agriculture and harmful for cotton productivity [1]. The growing abilities like boll size, seed per boll, reduction in fiber length that all effect the yield of cotton at maturation level due to increase in temperature [33]. The adverse impacts of climate change crop yield differ due to existence of harsh climatic conditions during growth stage. The marginal impact show that 1^0C in temperature during sowing season of cotton would increase yield by 1.65%. Since

cotton is heat tolerant crop, heating up of climate during sowing, flowering and picking stage will help increase in yield but further increase in temperature cause negative impact on productivity of cotton. As in Karachi due to increasing temperature and heat wave cotton yield may effecting. Another impact of higher atmospheric CO2 is that weeds will be growing more strongly as well. Precipitation has normally very small impact on yield of cotton. Cotton is grown by using various additional water sources in watered areas [37] show that precipitation reduces the yield of cotton. Temperature is beneficial and precipitation is harmful for cotton productivity. There is projection of high rainfall at the end of 21 century that is damaging to cotton productivity.
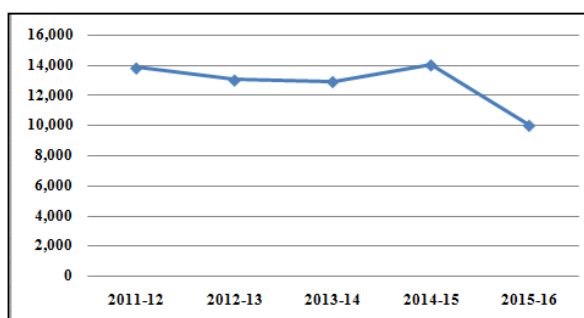
Source: Pakistan Bureau statistics (PBS)

Figure 6: production of cotton (000 bales) in Pakistan

Sugarcane

Sugarcane with high economic importance is growing in tropical and subtropical areas. Over the world in 2014 sugarcane grown in almost 27 million hectors in different countries [14]. Pakistan is ranked as 4th in the production of sugarcane [14]. Its production accounts for 0.6 percent in overall GDP of Pakistan. As it is not only produce as source of sugarcane but also using in industries as sweetener industries and residue (bagasse) may use in fuel production and electricity in sugar mills. Sugarcane need temperature range 30-32°C but above 34°C is unfavorable for crop growth [6-17]. Like other factors that controlling the bud development of sugar the rate of photosynthesis is also dependent on temperature, 8°C to a maximum of 34°C increase in temperature increase the photosynthesis efficiency. 14°C temperature in winter Cool nights and early morning and 20°C in summer significantly inhibit photosynthesis. By onset of mean day temperatures less than 21°C the peak growth of sugar-cane will have terminated because stalk is sensitive to temperature. Additional decrease in rainfall effect negatively on sucrose yield while increase in temperature of 2-3°C is beneficial for sucrose growth [21].

As a C4 crop it is studied that increasing carbon fertilization due to increase in temperature is beneficial effect of climate change [9]. Carbon consumption slow down by cool nights and sunny days, while photosynthesis may continue, thus enhancing sucrose accumulation. At high temperatures accumulation of sucrose is not favored as growth rate increases more than photosynthetic rates. Water logging is also a widespread phenomenon that drastically reduces the growth and survival of sugarcane and 18–64% reduction in cane yield due to water logging, depending on duration of water logging, plant growth stage. A shift in temperature due to climate change will effect on sugarcane production by attack of some of diseases, insects, and weeds. Sugar cane is effected by rainfall but resilience to temperature.
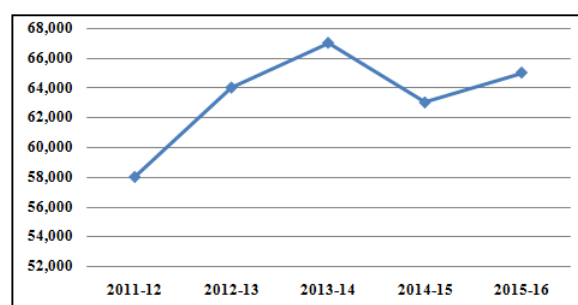
Source: Pakistan Bureau statistics (PBS)

Figure 7: production of Sugarcane (000 Tonnes) in Pakistan

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Maize

Most of the population in the world is relay on organic food which is rich with nitrogen but unfortunately the production of organic crops like maize is suppressing due to leaching effect in water bodies [23]. Maize contribution in agriculture is 2.2% and 0.4% to GDP of Pakistan. Maize is able to utilize solar and can tolerate relatively high temperature being a C4 plant up to a crucial factor. Development of plants and the yield may be reduced to 101 kg per hector per day when the temperature ranges up to 35°C in pollination. The most distressing factor that regulates the crop growth and definitive yields is high temperature. Maize can grow at the temperature range of 29°C and optimum maize production at 25°C. increase in temperature by 1 degree e.g. 30°C will reduce yield by 1% under rainfed condition [25]. Other studies show that increase 1°C will reduce 10% yield under normal condition [3].

Leaf growth in maize has been reported from 0 -350 ^{0}C, with decline at 35 -400^{0}C. Decline in photosynthesis and protein metabolism alter the aggregation, enzyme inactivation, repressed protein synthesis and its degradation was noted. Heat shock affects the endosperm development in maize and reduces grain yield due to disruption in cell division, sugar metabolism and starch biosynthesis. These studies suggest that due to increase in temperature maize not only effected by temperature stress but also become moisture sensitive. Overall the production of maize will decrease in future due to climate change scenario.
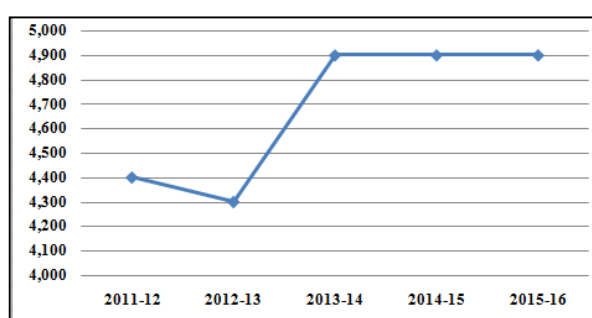
Source: Pakistan Bureau statistics (PBS)

Figure 8: production of Sugarcane (000 Tonnes) in Pakistan

Biotechnological adaptation options

To enhance the production of crops different adaptation techniques can use including soil health, conservation of water, diversity of livelihood, institution of local level [13]. All safety adaptations to effected groups provide application to efficient management of water, land and soil resources [30]. The production of cops is reducing due to changing in rainfall pattern, increase in temperature and attack of pests, weeds and diseases as a result of climate change. The productivity of crops per unit area can be improve by applying agricultural biotechnologies that enhance the crop yield and make crops able to cope harsh climatic conditions.

1. **Increase in area of land for yield**

To accomplish the need of nutrition crops over the world there are two options: first is to rise the production land and second is to enhance the farmland production of crops [10]. The second option is more practicable because of changing climate change dynamics and availability of arable land for the production. There are some modern and sustainable options to increase productivity including utilization of organic residue as a for nutrition of plants, efficient agricultural performs e.g. managing the landscape, crop revolution. The other factors to decrease the pest and diseases attack use of non-chemical traditional and local knowledge to farmers [2].

2. **Biotic stress resistant adaptations**

In this scenario of climate change the aim of biotechnology is to use fewer resources for sustainable productivity and capacity of crops. The production of stress that are resilient to insects, fungi and bacteria that are biotic stresses on crops can be developed by useful landscape practices increase breeding [41-2]. To produce protection against the insects, biotechnology provides the soil bacterium (Baccilus) that transform the genetic makeup of crops like maize and cotton that play a vital role in the pest control strategies. Studies shows that the productivity of crops by 11-12% e.g. canola and maize has been increasing by using biotic stress resistant genetically modified crops.

3. **A biotic stress resistant adaptation**

The challenges of climate change that impose negative impact on agriculture include shortage of land use and availability of water for irrigation. A biotic stain that poses negative impacts on environment due to climate change

are salinization, drought events, toxicity by chemicals and increase in temperature. Therefore, biotechnology provide adaptively measure to overcome a biotic stress like droughts and salinization.

Drought and salt resistant crops: production in forests is a programme of biotechnology that are developed by breeding. There is selection of some drought resistant plants like millet, cassava and sunflower that grown by conventional method in extreme climatic conditions to resist the harsh environmental conditions [26].

Molecular control mechanisms: In which production and regulation of genes that are stress tolerant. Some kinds of transgenic species produce that are metabolic, ion transported, antioxidants and less toxicants process to tolerate climatic stress. Other engineering method to cope these conditions include process of heat shock and heat resistant proteins. The activation and regulation of definite stress tolerant genes against abiotic stress[42]. The production of genetically modified enzyme like ADP ribose that can survive in harsh conditions. Studies shows that 44% of crop yield has been increased by using GM plants [5].

4. Agroecological and agroforest

The management approached of farmers to reduce the ecological and economical risks of crop damage by using different application to regenerate degraded soil and forests like mycoforestry and mycorestoration that resist extreme climatic conditions. To increase the ability of water uptake and fertility of soil technologies like mycorrhizal fungi and actinorhizal bacteria can be used [37].

Afforestation is also a contributing factor in lowering extreme temperature because forest and trees are carbon sinks that cause rainfall and reduce greenhouse gases. This is sustainable and effective mitigation method for climate change.

5. Biofertilizers

Biotechnology providing the new methods of using bio based fertilizer instead of artificial fertilizer like composting humus and animal manure. Also, production of non-leguminous plants to fix nitrogen in soil for the improvement in production of cereal crops[19]. For example, GM canola which is nitrogen efficient and economically beneficial for farmers [40].

Conclusion

The study shows that climate change prevalently effecting main agricultural crops. The result asses that wheat productivity is reducing due to short changes in temperature but long run increase effect positively on wheat production. Likewise, the changing in precipitation negatively effecting the wheat production in both short and long term. Rice production is initially grown by increasing temperature but further increase in optimal temperature will harmful for productivity. As wheat production reduce by increasing precipitation rice will not effected by this increasing phenomenon. Interestingly, the increase in precipitation does not harm the rice productivity. It has been obvious that the change in temperature and precipitation as variables of climate has a substantial negative impact on cotton productivity. As increase in temperature is advantageous but increase in precipitation is harmful for cotton productivity. Other crops like maize and sugarcane negatively effecting by increase in temperature. To overcome the problem of food security the mostly adaptive method is irrigation in rainfed areas that could be enhanced but farmers cannot afford. Farmers should adopt water and soil conservation methods for resilience to future climate change. The production of drought and heat resistant crops is effective method to cope harsh conditions of climate change.

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Growth and Yield Response of Mungbean under the Influence of Nitrogen and Phosphorus Combination Levels

Sanaullah Jamro[1], Muhammad Ali Ansari[1], Majid Ali Jamro[2], Muhammad Irfan Ahmad[3],
Waheed Ahmed Siddiqui[4], Shahid Ahmad Junejo[5], Soualihou Soualiou[3], Sikandar Ali Jamro[3]

[1]Department of Agronomy, Sindh Agricultural University, Tando Jam Pakistan.
[2]Department of Plant Breading and Genetics, Shaheed Zulfiquar Ali Bhutto, Agriculture Collage Dokri
[3]School of Agronomy, Anhui Agriculture University Hefei - 230036, Anhui Province, P.R. China
[4]Agriculture engineering Department, Anhui Agricultural University, Hefei -230036, Anhui Province, P.R. China
[5]School of Tea and Food Science Technology, Anhui Agricultural University, Hefei -230036,
Anhui Province, P.R. China

ABSTRACT

The results of the experiment showed that various combination of Nitrogen and Phosphorus (NP) levels significantly affected crop parameters. The maximum crop stand m^{-2} (128.7), plant height cm (59.54), number of branches $plant^{-1}$ (12.74), number of pods $plant^{-1}$ (33.32), number of seeds $plant^{-1}$ (376.2) seed weight $plant^{-1}$ (17.61 g) seed index (32.05 g) and seed yield (2290.0 kg ha^{-1}) were found with the application of NP combination level of 50-75 kg ha^{-1} . Whereas, for varieties, V1 i.e. AEM-96 surpass in all parameters as compared to V_2 i.e. NM-94, which gave maximum (120.11) crop stand m^{-2}, maximum (52.55 cm) plant height, 10.67 plant m^{-2}, maximum (24.03) branches $plant^{-1}$, maximum (15.07) pods $plant^{-1}$, maximum (30.01 g) seed weight $plant^{-1}$ and maximum (2439.2 kg ha^{-1}) seed yield. Among the interactions the highest seed yield was recorded in variety AEM-96 with NP combination level of 50-75 kg ha^{-1} followed by the interaction of variety AEM-96 with NP combination of 50-50 kg ha^{-1}, whereas lowest seed yield kg ha^{-1} was recorded variety NM-94 with NP combination 25.00 kg ha^{-1}The variety AEM-96 under NP combination level 50-75 kg ha^{-1} perform better and gave highest yield 2439.2 kg ha^{-1}.

KEYWORDS: Fertilizer; Yield; agronomic parameters; Mungbean

INTRODUCTION

Mungbean or green gram *(Vignaradiata. L.)* is commonly known as mung. It belongs to family leguminoceae. It is an important pulse crop having high nutritional value and low cost protein, containing fair amount of protein (24.21 %), vitamin A (80 units), carbohydrates 69.30 % and high caloric value. It restores the fertility of soil by fixing atmospheric nitrogen through root nodules. In Pakistan, it is grown on as area of about 239.2 thousand hectares with the total annual production of 115.4 thousand tones. In Sindh province the mungbean is cultivated on an area of 11.1 thousand hectares with the annual production of 5.5 thousand tones (Pakistan Statistical Year Book, 2003).[1]

The production of mung is still very low as compared to other developed countries due to various constraints. Among them, appropriate fertilizer application plays an important role for the growth and yield of the mungbean crop. According to Prasad *et al*. (1998) [2] that potassium increased the total biomass and plant protein in mungbean. Malik *et al*. (2000) [3] reported that maximum seed index, grain yield and protein were obtained satisfactory from the plots where inoculated seed was grown with phosphorous applied at the rate of 50 kg ha^{-1}. Thus, increase in grain yield was attributed to increase the number of pods per plant, number of grains per pod and heavier grain weight. Abd-El-Lateef *et al*. (1998a) [4] noted that the application of urea with Zn increased the number of branches per plant and protein content in seeds while, the greatest numbers of leaves per plant were produced from the combined application of urea with Mn, Zn and Cu. Further, Bamel*et al*. (2002) [5] reported that combination of N, P, K and Zn fertilizer at the recommended rate resulted in better plant growth as well as reduced nematode damage individually, and application of di-ammonium phosphate at higher rate resulted in the better plant growth. Being a leguminous crop, mungbean does not need a high dose of nitrogenous fertilizer however 20 kg N ha^{-1} as a starter dose was found for good crop growth and accelerated nodulation. Rajender*et al*. (2003) [6] also observed that grain yield at mungbean increased with nitrogen at the rate of 20 kg ha^{-1} over control and crop

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Corresponding Author: Sikandar Ali Jamro, School of Agronomy Anhui Agriculture University Hefei - 230036, Anhui Province, P. R. China

exhibited better cost benefit ratio however Patel *et al.* (2003) [7] with careful investigations found that 10 kg N ha^{-1} was enough better mungbean crop production.

Field crops require 16 essential nutrients to grow normally. Carbon, Hydrogen and oxygen are derived from the air comprise greater than 90 per cent of the fresh plant tissue. Macronutrients, needed in large amounts, derived from the soil are nitrogen, phosphorus, potassium, sulphur, calcium, and magnesium. Legumes are the exception because they fix N from the air. With a few exceptions, Ca and Mg are not limiting because of the nature of the soils. The soil supply of N, P, K, and S is often supplemented by fertilizers and manure. The remaining essential nutrients, derived from the soil, are referred to as micronutrients, because they are needed in small amounts (Sharma *et al.,* 2003). [8]

For plant growth and development, the role of essential nutrients like N, P and K is of prime importance. Nitrogen has important role in the physiological process of the plant and its appropriate rate at the time of sowing leads to rapid leaf area development, prolongs leaves life, improves leaf area duration (LAD), after flowering causing increase in overall assimilation rate, thus contributing to increased seed yield. Phosphorus helps in energy transfer reactions and is important for the growth of roots and branches of the plants, whereas its deficiency restricts both top and root growth. With severe deficiency, the root system is poorly developed and stems are thin and erect with few branches and small, narrow leaves (Tahir *et al.,* 2003)[9]. The Potassium has key role in increasing the plant vigor, straw length and helps in speedy healing of wounds caused by insects or hail and wind. Deficiency of potassium also causes in yield reduction due to stunt growth of plants and seeds of small size (Balasubramaniyan and Palaniappan, 2001) [10]. The growers mostly apply nitrogenous, phosphatic and to some extent potassic fertilizers. However, the recent studies revealed that the application of P is an essential element for maintaining the soil fertility. Deficiency of P is attributed to removal by high yielding crop varieties, use of high doses and pure forms of NPK nutrient fertilizers and intensive system of cultivation (Anonymous, 2003).[1]

Generally in rainfed areas, no fertilizers and manures are applied; in some places farmers may apply a small amount of F.Y.M. (e.g. 8-15 t ha^{-1}) 3-4 weeks before sowing. In irrigated areas, a small amount of mineral fertilizer (10-15 kg ha^{-1} N, 20-30 kg ha^{-1} P_2O_5) is either placed in the seed furrow or broadcast and mixed in the soil. Under improved cultivation practices: irrigated, basal application of 18-20 kg ha^{-1} N, 40-50 kg ha^{-1} P_2O_5, plus K, Zn, S if required; un-irrigated, basal application of 10-15 kg ha^{-1} N and 20-30 kg ha^{-1} P_2O_5, followed by a foliar spray of 2 % urea at pod development. Di-ammonium phosphate, urea and single super phosphate are commonly used (Zeven, 1998) [11].

Singh *et al.* (2001) [12]cultivated *Vignaradiata* , cv. K-851, in 30, 60 or 90 kg P_2O_5 and 5, 10 or 15 ppm Zn, compared with untreated controls. P and Zn application increased the seed protein, N and P contains higher P_2O_5 increased seed yield considerably. Thakur and Giri (2001)[13] applied 0, 25, 50 or 75 kg P_2O_5 ha^{-1} as Single Super Phosphate or Di-ammonium Phosphate is mungbean, *Vignaradiata*(L). seed and straw yields were not significantly affected by P source, and seed yield average of 0.91 , 1.00, 1.24 and 1.13 t ha^{-1} at the 4 P rates, respectively. P uptake was also highest with 50 kg P_2O_5.

Keeping in view the importance of mungbean as pulse crop and its management in relation to chemical fertilizers, the present experiment was conducted to examine the growth and yield response of mungbeanunder the influence of NP combination levels under agro-ecological conditions of Tandojam.To evaluate the impact of NP levels on growth, yield and yield components of mungbean,To determine the most appropriate NP level for maximizing mungbean yield.

MATERIALS AND METHODS

An was carried out to evaluate the growth and yield response of mungbean under the influence of NP combination levels during the year 2013 at the Experimental Area of Oilseeds Section, Agriculture Research Institute, Tandojam. The detail of the experiment was as under:

Experimental design: Randomized Complete Block Design (RCBD)

Replications: 03

Plot size:3 x 3 m = (9 m^2)

Treatment Two factor (A and B)

Factor –A = Varieties = 02

V$_1$= AEM-96   V$_2$= NM-94

Factor-B **NP Fertilizer Combinations = 07**

F0= (Control) 0-0 NP,   F1= 25-0 NP kg ha^{-1},   F2=25-50NP kg ha^{-1}

F3= 25-75 NP kg ha^{-1} ,   F4= 50-0 NP kg ha^{-1},   F5=50-50 NP kg ha^{-1}

F6= 50-75 NP kg ha^{-1}

Treatment combinat ions

$T_1 = V_1 F_1$	$T2 = V_1 F_2$	$T3 = V_1 F_3$	$T4 = V_1 F_4$
$T5 = V_1 F_5$	$T6 = V_1 F_6$	$T7 = V_2 F_1$	$T8 = V_2 F_2$
$T9 = V_2 F_3$	$T10 = V_2 F_4$	$T11 = V_2 F_5$	$T12 = V_2 F_6$

Land preparation

A suitable piece of land which was lying fallow was ploughed up by cross-wise disc plough, after soaking dose, when the land came in condition the seedbed was prepared by using cultivator (cross-wise) and rotavator. Thereafter, clods were crushed completely by clod crusher followed by planking.

The sowing was done with the help of single coulter hand drill in lines. The treatments were managed in such a way to discriminate the plots or treatments and replications easily the channels and bunds were prepared to facilitate the irrigation process and further monitoring of the crop against any pest problem. The cultivated mungbean two varieties AEM-96 and NM-94 were used in this experiment. The experiment comprised of the following treatments. Phosphorus fertilizer was applied in the form of Single Super Phosphate. As per schedule of treatments, all the Phosphorus in the form of SSP (18 %P_2O_5) was applied at the time of sowing. A uniform dose of nitrogen was also applied to keep the experimental soil adequate in this essential nutrient. Nitrogen was applied in the form of Urea (46%) in three splits. The first dose of nitrogen (1/3 N) was applied at the time of sowing, the second (1/3 N) at the first irrigation and the final (1/3 N) at the time of third irrigation. Irrigations were applied as per the schedule. Potassium fertilizer was not applied, as soil was adequate in K. The row spacing maintained at 75 cm apart and distance between plants was 30 cm. The recommended cultural practices were performed in all the subplots. Irrigations were applied according to the requirements of the crop depending upon the soil moisture conditions. In all two irrigation were applied after 28-days and 50-days after sowing.

Observations recorded

Five plants in each treatment were selected at random for all the observations, these plants were tagged and numbered separately. The observations were recorded on the following parameters.
1. Crop stands m^{-2}
2. Plant height (cm)
3. Branches $plant^{-1}$
4. Pods $plant^{-1}$
5. Seeds $plant^{-1}$
6. Seed weight $plant^{-1}$ (g)
7. Seed index (1000 seed weight, g)
8. Seed yield (kg ha^{-1})

RESULTS

The treatments including on 07 NP fertilizer combination viz. F0=0-0 (Control), F1=25-00, F2=25-50, F3=25-75, F4=50-00, F5=50-50 and F6=50-75 kg ha^{-1}. The observations were recorded on the characters of economic importance such as: Crop stand m^{-2}, plant height (cm), branches $plant^{-1}$, pods $plant^{-1}$, seeds $plant^{-1}$, seed weight $plant^{-1}$, seed index (1000 seed weight, g) and seed yield kg ha^{-1}. The data recorded on the above characters are presented in Table-1 to 8 and their analyses of variances are given as Appendices I to VIII. The findings of results are interpreted in the following paragraphs:

Crop Stand m^{-2}

Crop stand is the major yield component, the results regarding their mean crop stand m^{-2} of mung bean as affected by NP combination levels are presented in Table-1 and its analysis of variance as Appendix-I. The results revealed that there was significant (P<0.01) effect on crop stand m^{-2} due to different NP combination levels.

It is apparent from Table-1 that plant stand m^{-2} were significantly affected (P<0.01) maximum 128.7 m^{-2} were recorded when mung bean crop was fertilized with highest NP combination level 50-75 kg ha^{-1} followed by NP combination of 50-50 kg ha^{-1}. The mung bean crop when applied lower NP combination i.e. 25-00 kg ha^{-1} which recorded lower crop stand m^{-2}. Further results revealed that variety AEM-96 recorded better crop stand m^{-2} (120.11) than variety NM-94, furthermore, among the interactions of NP combination and varieties. Data displayed the

maximum plant stand m^{-2}(130.00) in the interaction of 50-75 x AEM-96 whereas, minimum plant stand m^{-2} 103.33 was recorded in the interaction of 25.00 NP kg ha^{-1} x NM-94.

Table 1. Mean crop stand m^{-2} of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha-1						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	106.67	116.00	122.00	120.00	126.00	130.00	120.11-A
V2= NM-94	103.33	114.00	118.33	117.33	122.33	127.33	117.11-B
Mean	105.0-E	115.0-D	120.2-C	118.7-C	124.2-B	128.7-A	-

S.E. for Treatment Mean					
	Varieties	Fertilizer	V x F	S.E. / Plot	C.V %
	0.2750	0.4763	0.6736	1.1666	0.98 %
Cd-1	0.7800	1.3970	-	-	-
Cd-2	1.0700	1.8999	-	-	-

Plant height (cm)

Plant height is the major growth character which is reflected by the soil fertility under which crop grown. The results regarding the plant height of mungbean as affected by different phosphorus levels are recorded in Table-2 and the analysis of variance as Appendix-II. The analysis of variance suggested that there was significant ($P<0.01$) effect on the plant height of mungbean due to different NP combination levels.

It is apparent from the data in Table-2 that plant height was significantly ($P<0.01$) maximum (59.54 cm) when the mungbean crop was fertilized with highest NP combination of 50-75 kg ha^{-1}, closely followed by NP combination of 50-50 kg ha^{-1} with average plant height of 56.47 cm. The mung bean crop when fertilized with relatively lower NP combination of 25-50 kg ha^{-1}, the average plant height was considerably decreased to 47.70 cm, respectively. The mungbean crop under control plots, where phosphorus was not applied resulted in minimum plant height of 37.58 cm. It was noted that each increased phosphorus level up to 75 kg ha^{-1} substantially improved the plant height. Further results demonstrated that taller plants 52.55 cm were noted in variety AEM-96 than variety NM-94. Furthermore, results revealed that taller plants 61.22 cm were displayed by the interaction of NP combination of 50-75 kg ha^{-1} and variety AEM-96 and smaller plants 35.90 cm were recorded among the interaction of NP combination and varieties i.e. 25.00 NP kg ha^{-1} and variety NM-94.

Table 2. Mean plant height (cm) of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha^{-1}						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	39.27	48.86	55.50	52.66	57.82	61.22	52.55-A
V2= NM-94	35.90	46.53	53.39	51.93	55.12	57.86	50.12-B
Mean	37.58-F	47.70-E	54.45-C	52.29-D	56.47-B	59.54-A	-

S.E. for Treatment Mean					
	Varieties	Fertilizer	V x F	S.E. / Plot	C.V %
	0.1778	0.3079	0.4355	0.7543	1.47 %
Cd-1	0.5100	0.9032	-	-	-
Cd-2	0.7000	1.2280	-	-	-

Number of branches plant^{-1}

Number of branches is one of the most important growth character as well as yield contributing trait which resulted that more pods plant[-1]. The data pertaining to the number of branches plant[-1] of mung bean as affected by different NP combination are presented in Table-3 and the analysis of variance is shown as Appendix-III. The results of the analysis of variance suggested that the number of branches plant[-1] significantly ($P<0.01$) affected due to different NP combination levels.

The mung bean crop fertilized with highest NP combination produced significantly ($P<0.01$) highest number of branches (12.74) plant[-1], closely followed by NP combination of 50-50 kg ha^{-1} with 12.04 branches plant[-1] and variety AEM-96 displayed maximum branches 10.67 plant[-1] than variety NM-94. Further among the interaction of NP combination and varieties, the maximum branches (13.27) plant[-1] were recorded in the interaction of 50-75 NP kg ha^{-1} and AEM-96 minimum number of branches (5.21) plant[-1] were noted in the interaction of 25.00 NP kg ha^{-1} and variety NM-94. However, in plots where the mung bean crop was left without NP had lowest number of branches (6.15) plant[-1].

Table 3. Mean number of branches plant[-1] of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha^{-1}						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	7.09	9.21	11.45	10.51	12.48	13.27	10.67-A
V2= NM-94	5.21	8.36	10.70	9.35	11.60	12.22	9.57-B
Mean	6.15-E	8.78-D	11.08-B	9.93-C	12.04-AB	12.74-A	-

S.E. for Treatment Mean					
	Varieties	Fertilizer	V x F	S.E. / Plot	C.V %
	0.1497	0.2593	0.3667	0.6348	6.28 %
Cd-1	0.4300	0.7601	-	-	-
Cd-2	0.5900	1.0330	-	-	-

Number of pods plant[-1]

Number of pods plant[-1] is mainly associated with the number of branches plant[-1] and generally it is assumed that more the number of branches plant[-1], greater will be the number of pods. The results regarding the number of pods plant[-1] of mungbean as affected by different fertilizer NP combination are given in Table-4 and the analysis of variance as Appendix-IV. The analysis of variance illustrated that the number of pods plant[-1] was affected significantly ($P<0.01$) due to different NP combination levels.

Variety AEM-96 recorded maximum number of pods plant[-1] than variety NM-94. Further the results among the interactions of NP combination and varieties, the highest number of pods plant[-1] 34.63 were obtained in the interaction of NP combination of 50-75 kg ha^{-1} and variety AEM-96 and minimum number of pods plant[-1] 14.40 were recorded in the interaction of 25.00 kg ha^{-1} and variety NM-94. It is obvious from Table-4, that the mung bean crop fertilized with highest NP combination of 50-75 kg P ha^{-1}, produced significantly ($P<0.01$) maximum number of pods plant[-1], closely followed by NP combination of 50-50 kg ha^{-1} where the crop bearing average of 33.32 pods plant[-1], whereas, the minimum number of pods plant[-1] (15.80) was recorded in mungbean plants that received zero NP fertilizers (control). It was noted that P application had positive effect on the number of pods, and increase in P levels, significantly improved the number of pods.

Table 4. Mean number of pods plant[-1] of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha^{-1}						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	17.19	19.09	23.02	21.30	28.92	34.63	24.03-A
V2= NM-94	14.40	16.61	20.37	18.68	27.66	32.00	21.62-B
Mean	15.80-F	17.85-E	21.69-C	19.99-D	28.29-B	33.32-A	-

S.E. for Treatment Mean				
Varieties	**Fertilizer**	**V x F**	**S.E. / Plot**	**C.V %**
0.1832	03173	0.4487	0.7772	3.41 %
Cd-1	**0.5300**	0.9306	-	-	-
Cd-2	**0.7300**	1.2650	-	-	-

Number of seeds plant[-1]

Number of seeds plant[-1] is an important character that had direct effect on the seed yield plant[-1] and per unit area. Generally, it is assumed that more the number of pods, greater will be the number of seeds plant[-1]. The results regarding the number of seeds plant[-1] of mung bean as affected by different NP combination levels are given in Table-5, its analysis of variance as Appendix-V. The analysis of variance suggested that the number of seeds plant[-1] was affected significantly (P<0.01) due to different fertilizer NP combination levels.

It can be seen from Table-5 that highest NP combination level revealed in significantly (P<0.01) highest (376.2) number of seeds plant[-1], closely followed by NP combination level of 50-50 NP kg ha^{-1} where the mung bean plant bearing an average of 362.1 seeds plant[-1], however, the lowest (184.4) number of seeds plant[-1] was counted in mung bean plants which received no phosphorus fertilizers (control).

The results further indicated that NP application positively affected the number of seeds, and each increment in NP levels, significantly increased the number of seeds. Further results revealed that maximum number of seeds plant[-1] 301.42 was recorded by variety AEM-96 than variety NM-94, among the interaction of NP combination and varieties, the highest number of seeds plant-1 (377.4) were recorded by the interactions of 50-75 NP kg ha^{-1} and variety AEM-96 whereas, minimum seeds plant-1 182.7 were noted in the interaction of 25.00 NP kg ha^{-1} and variety NM-94.

Table 5. Mean number of seeds plant[-1] of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha^{-1}						
	F1= 25-00	**F2= 25-50**	**F3= 25-75**	**F4= 50-00**	**F5= 50-50**	**F6= 50-75**	**Mean**
V1= AEM-96	186.2	239.9	327.6	312.3	365.0	377.4	301.42-A
V2= NM-94	182.7	234.2	323.4	306.3	359.2	374.9	296.80-B
Mean	184.4-F	237.0-E	325.5-C	309.3-D	362.1-B	376.2-A	-

S.E. for Treatment Mean				
Varieties	**Fertilizer**	**V x F**	**S.E. / Plot**	**C.V %**
0.2022	0.3502	0.4953	0.8579	0.29 %
Cd-1	**0.5919**	1.0270	1.453	-	-
Cd-2	**0.8064**	1.3960	1.974	-	-

Weight of seed plant-1 (g)

The results regarding the weight of seeds plant[-1] of mungbean as affected by different NP s combination levels are shown in Table-6 and the analysis of variance as Appendix-VI. The results of the analysis of variance described that the weight of seeds plant[-1] was influenced significantly (P<0.01) due to different NP combination levels.

The results shown in Table-6, indicated that the highest (17.61 g) weight of seeds plant[-1] was obtained from the mungbean crop which fertilized with highest NP combination, closely followed by 16.39 g average weight of seeds plant[-1] recorded from the crop receiving 50-50 NP combination kg ha^{-1}. However, the lowest (10.82g) weight of seeds plant[-1] was noted in mung bean plants which were left unfertilized with P (control). The results further showed that there was a linear effect of increasing P levels on weight of seeds plant[-1] and there was consecutive improvement in weight of seeds.

Similarly, like number of seeds plant[-1] variety AEM-96 displayed maximum seeds weight plant[-1] (15.07 g) than variety NM-94. Among the interactions of NP combination and varieties the highest seeds weight plant-

[1](18.72g) were recorded in the interaction of 50-75 NP kg ha^{-1} and variety AEM-96 whereas lowest seeds weight plant[-1] (10.35 g) were recorded in the interaction of 25.00 NP kg ha^{-1} and variety NM-94.

Table 6. Mean seeds weight plant[-1] (g) of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha^{-1}						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	11.29	13.23	15.33	14.46	17.52	18.72	15.07-A
V2= NM-94	10.35	12.21	14.46	12.74	15.27	16.50	13.59-B
Mean	10.82-E	12.76-D	14.90-C	13.50-D	16.39-B	17.61-A	-

S.E. for Treatment Mean					
	Varieties	Fertilizer	V x F	S.E. / Plot	C.V %
	0.1141	0.1976	0.2794	0.4837	3.38 %
Cd-1	0.3340	0.5492	-	-	-
Cd-2	0.4550	0.7872	-	-	-

Seed index (1000 seeds weight, g)

Seed index value is generally considered as quantity and quality character in grain seed producing crops and particularly considered. The data pertaining to seed index of mungbean as affected by different NP combination are recorded in Table-7 and the analysis of variance as Appendix-VII. The analysis of variance illustrated that the seed index was influenced significantly ($P<0.01$) under the effect of different NP combination levels.

It is apparent from the data in Tabsle-7 that seed index value was significantly ($P<0.01$) higher (32.05 g) when the crop received highest NP combination level of 50-75 kg ha^{-1}, closely followed by NP combination level of 50-50 kg ha^{-1} resulting seed index value of 31.07g. The seed index value under control plots was minimum (27.03 g), where zero NP combination was applied to mung bean crop. It was noted that each increased phosphorus level upto 75 kg ha^{-1} substantially improved the seed index value. The situation suggested that 50-75 NP combination kg P ha^{-1} was an optimum level for producing economical mung bean production.

Further data indicated that highest seed index 30.01 g was displayed by variety AEM-96 than variety NM-94. Among the interactions the maximum seed index 32.47 g was displayed by the interaction of 50-75 NP kg ha^{-1} and variety   AEM-96 were as minimum seed index 26.46 was recorded in the interaction of 25.00 NP kg ha-1 and variety NM-94.

Table 7. Mean seed index (1000 seeds weight, g) of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	NP Fertilizer combination levels kg ha^{-1}						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	27.61	28.52	30.52	29.40	31.56	32.47	30.01-A
V2= NM-94	26.46	27.41	29.46	28.54	30.58	31.64	29.01-B
Mean	27.03-F	27.97-E	29.99-C	28.97-D	31.07-B	32.05-A	-

S.E. for Treatment Mean					
	Varieties	Fertilizer	V x F	S.E. / Plot	C.V %
	0.1331	0.2305	0.3260	0.5648	1.91 %
Cd-1	0.3896	0.6763	-	-	-
Cd-2	0.5308	0.9192	-	-	-

Seed yield (kg ha^{-1})

The seed yield ha^{-1} is a character of ultimate important and most of the studies focus to reach its highest NP combination under certain treatments or crop varieties. The results regarding the seed yield ha^{-1} of mungbean as affected by different NP combination levels are recorded in Table-8 and the analysis of variance as Appendix-VIII. The analysis of variance illustrated that the seed yield ha^{-1} was significantly (P<0.01) affected due to different NP combination levels.

It can be seen from the results presented in Table-8 that the highest seed yield (2290.0 kg ha^{-1}) was obtained from the mungbean crop fertilized with highest NP combination level of 50-75 kg P ha^{-1}, while it was closely followed by an average seed yield of 2058.0 kg ha^{-1} was recorded from the crop receiving NP combination of 50-50 kg ha^{-1}. However, the minimum seed yield of 1153.0 kg ha^{-1} was recorded from the plots receiving zero phosphorus fertilizer (control). The results further suggested that there was a linear effect of increasing P levels upto 75 kg ha^{-1} on seed yield ha^{-1}. Further the results revealed that maximum seed yield kg ha^{-1} 1832.80 was recorded by the variety AEM-96 than variety NM-94. Among the interactions the results reported that highest seed yield 2439.2 kg ha^{-1} was recorded by the interaction of 50-75 NP kg ha^{-1} and variety AEM-96, but lowest seed yield kg ha^{-1} 1103.8 was noted in the interaction of 25.00 NP kg ha^{-1} and varieties.

Table 8. Mean seed yield (kg ha^{-1}) of mung bean varieties as affected by different NP fertilizer combination levels.

Varieties	Fertilizer NP combination levels kg ha^{-1}						
	F1= 25-00	F2= 25-50	F3= 25-75	F4= 50-00	F5= 50-50	F6= 50-75	Mean
V1= AEM-96	1202.4	1571.0	1858.6	1686.7	2241.9	2439.2	1832.80-A
V2= NM-94	1103.8	1354.2	1639.1	1559.1	1875.9	2140.3	1611.73-B
Mean	1153.0-F	1463.0-E	1748.0-C	1622.0-D	2058.0-B	2290.0-A	-

S.E. for Treatment Mean					
	Varieties	Fertilizer	V x F	S.E. / Plot	C.V %
	10.3681	17.9582	25.3967	43.9883	2.55 %
Cd-1	30.3518	52.6700	74.4900	-	-
Cd-2	41.3483	71.5900	101.2000	-	-

DISCUSSION

In Pakistan, mostly nitrogenous fertilizers are applied, and less attention is given to the significance of phosphatic and potassic fertilizers; while the application of P is essential for maintaining the soil fertility. Phosphorus (P) is essential for plant growth it stimulates growth of young plants, giving them a good and vigorous start. Phosphorus management and nutrition has both economic and environmental implications. Phosphorus exists in soils in organic and inorganic forms. Organic forms of P are found in humus and other organic material. The process is most rapid in warm, well-drained soils. Research shows that 1% of the total soil organic phosphorus is mineralized per year during mung bean production. However, since initial levels are low, and plant uptake is only one possible fate of the mineralized phosphorus, the contribution by mineralization to plant available phosphorus is small (Anonymous, 2003)[1].

In the present study, seven NP combinations 0-0, 25-00, 25-50, 25-75, 50-00, 50-50 and 50-75 kg ha^{-1} were examined to evaluate their effect on seed germination, plant height, number of branches plant^{-1}, number of pods plant^{-1}, number of seeds plant^{-1}, weight of seed plant^{-1}, 1000 seed weight and seed yield kg ha^{-1}. The results showed that application of NP combination had significant (P<0.01) effect on all the above growth and seed yield contributing characters of mungbean and there was a linear and significant (P<0.01) effect of increasing P levels and highest NP combination of 50-75 kg ha^{-1} recorded maximum values for all the growth and yield components, closely followed by 50-50 NP combination ha^{-1}.

Kumar *et al.* (2002a) [14] examined the effect of different P levels in the research studies In conducted in India on mungbean .They applied P at 0, 20, 40 and 60 kg ha^{-1} There report are that number of branches, number of pods plant^{-1}, number of seeds pod^{-1}, 1000-seed weight and straw yield increased with increasing rates of P, whereas grain yield was highest under 40 kg P ha^{-1}. This little contradiction may have associated with the fertility of the

experimental soil and overall environmental conditions, because our soils have severe deficiency of N followed by P. Similarly, in another study in India, Lukoki *et al.* (2000)[15] applied phosphorus at 0, 25, 50, and 75 kg ha^{-1} levels and obtained higher grain yield (764 kg ha^{-1}) with 25 kg P ha^{-1}, while in present study the yield level was well higher (>2290 kg ha^{-1}).under 50-75 kg ha^{-1}.

Whereas variety AEM-96 recorded maximum seed yield 1832.80 kg ha-1 than variety NM-94. But among the interactions the maximum seed yield 2439.2 kg ha^{-1} was recorded by interaction of NP kg ha^{-1} 50-75 and variety AEM-96 followed by the interaction NP kg ha^{-1} 50-50 x variety AEM-96 i.e. 2241.9 kg ha^{-1} whereas minimum seed yield kg ha^{-1} 1103.8 was recorded by the interaction of NP kg ha^{-1} 25.00 and variety NM-94.

Parsad *et al.* (2000) [16]applied phosphorus at 30, 60 and 90 kg ha^{-1} in mungbean and obtained highest seed yield with 60 kg P ha^{-1}, while Srinivas and Mohammad (2002) [17]concluded that plant height, number of branches and leaves plant^{-1}, number of pods plant^{-1}, pod length, number of seeds pod^{-1}, 1000-seed weight, and seed and haulm yield generally increased with increasing rates of P. In another study Thind *et al.* (2002)[18] recommended 90 kg P ha^{-1} for higher mungbean yield, while Patel *et al.* (2003)[7] obtained higher mungbean yields from P application upto 60 kg ha^{-1}.

Considerable research in Pakistan on the similar aspects has also been conducted. Nadeem *et al.* (2004)[19] reported that application of 60 kg P$_2$O$_5$ significantly increased the seed yield, while Goswami (2006) [20]examined 50, 75 and 100 kg ha^{-1} P levels and reported that 100 kg P ha^{-1} along with 75 kg N resulted in better growth and higher values in yield contributing characters.

The comparison of the values for growth and yield components obtained in the present investigation and results reported from other parts of the world on the similar aspect, it can be easily justified that 75 kg P ha^{-1} with 50 kg N ha^{-1} remained mostly optimum for getting higher economical mung bean yields in Tandojam conditions.

CONCLUSIONS

It was concluded from the findings of the present study that there was a linear and significant (P<0.01) effect of NP combination levels on the growth and seed yield kg ha^{-1}. The above trend of effectiveness makes it obvious that NP combination levels 50-75 kg ha^{-1} performed better seed yield production of variety AEM-96 under agro-climatic conditions of Tandojam.

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Rational Emotive Behavior Therapy Effective for the Features of Social Anxiety among University Students

Amna Aurooj, Tazvin Ijaz

Clinical Psychology Unit, Government College University, Lahore, Pakistan.

ABSTRACT

Social Anxiety is evidently becoming an increasing problem in this socially modern era as a person's self–acceptance is getting tilted towards others point of view. This anxiety is becoming quite prevalent among university students where it inculcates negative thinking, emotion and behavior pattern. This study targeted effectiveness of Rational Emotive Behavior Therapy on the features of social anxiety among university students.

Method: A pre/post test quasi experimental design was used where a sample of 10 referred clients with manifesting features of social anxiety, who scored above the cut-off score of 19 on Social Phobia Inventory (SPIN) was selected [20]. Clients were randomly assigned to Experimental and control groups (5 clients per group). Treatment consisted of 8 sessions compromising of Rational Emotive Behavior Therapy, with a follow-up of 2 sessions.

Results: Post-test via Social Phobia Inventory (SPIN) suggested effective decrease in the symptoms of social anxiety for experimental group as compared to the control group.

Conclusion: This study provides evidence that Rational Emotive Behavior Therapy can effectively alleviate social anxiety particularly the features of fear, avoidance and other physiological symptoms that causes hindrance for a person's well-being.

KEY WORDS: Social anxiety; Rational Emotive Behavior Therapy, University Students

1. INTRODUCTION

Social anxiety is described as a feeling of extreme anxiety and fear regarding social situations which may impair the daily functioning of individuals and may compel them where to avoid the scrutiny of others [1]. The prevalence of social anxiety disorder is different across the studies due to sampling, assessment strategies, applied diagnostic method, and cultural norms. In western culture, prevalence of the disorder is measured to be in higher rates than the eastern culture. One of the reasons could be the cultural aspects of the society and its associated self construct [2]. Distinct prevalence of social anxiety in the eastern cultures ranging from 0.5% is reported rather than in a western culture which is 16% in general population [3]. Studies suggested that the age of onset is typically in adolescence than in adulthood. Early onset at the age of 11 years has been reported in about 50% and by the age of 20 years in about 80% of the individuals [4].

Risk factors implicated for the development of the disorder include deficits in social skills and negative peer status, along with family-related factors including parental psychopathology, heritability estimates and temperament, anxiogenic parenting, and transmission of interpretation bias [5]. Other studies have also reported about the influence of genetic and biological dispositions, cognitive factors, parental and peer relations, deficits in performance, modes of learning, and cultural factors are responsible for the development of the disorder [6]. The factors which influence the social anxiety among young individuals are social interactions particularly in educational institutions. These social interactions help the young individual in learning educational and life skills particularly through peers. But if judged negatively, it may develop anxiousness in the young individuals [7]. Their daily functioning might get disturbed as they could develop problems in class participation, making friends, attending classes or not even taking the classes' altogether [8].

Social anxiety is characterized as a complicated and impenetrable vicious cycle of negative expectations of social situations in which students befall. Effective interventions are needed to address a variety of factors, notably negative thinking, poor social skills even including physical appearance features as well [9]. A plethora of studies conducted for finding out the effective treatments for social anxiety disorder in adolescents and young adults suggested Cognitive Behavior Therapy [10], Mindfulness [11], Attention Training [12] and Social Skills Training (SST) [13] to be efficient in dealing with Social Anxiety Disorder. Literature has suggested that social anxiety also possesses the emotional dysregulation as well. These emotional dysregulation led to significantly lower levels of fixed

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Corresponding author: Amna Aurooj, Institute of Clinical Psychology, University of Management and Technology, Lahore, Pakistan. E-mail: amna.aurooj@gmail.com

beliefs about anxiety and other kinds of maladaptive beliefs and discussing about families of emotion regulation processes including selection and modification of situation, attentional deployment, change in cognition, and response modulation [14].

Rational Emotive Behavior Therapy (REBT) is considered to be a new dimension in the treatment of psychological disorders as it underpins cognitive, emotional and behavioral deregulation [15]. It regulates from recognizing, appraising and assessing one's irrational self-deprecating beliefs and retaliates against them by disputing those beliefs and formulating positive change within oneself. REBT was found effective on the shyness among Nigerian university students, improving their self-efficacy before graduation [16]. REBT in group form was found to be an effective strategy in dealing with the distress caused by paranoia [15]. A study conducted to study the impact of REBT on Indian adolescents suffering from conduct disorder showed significant results as well [17]. Effectiveness of Group Rational Emotive Behavior Therapy (REBT) in the treatment of shyness in Pakistani female college students was also observed [18]. Rational Emotive Behavior Therapy also proved effective in disputing irrational beliefs of persons with substance used disorders [19]. These studies proclaimed that Rational Emotive Behavior Therapy has a potential to be effective for many psychological problems while honoring the diversity among individuals.

The main objective of the current study was to find the effectiveness of Rational Emotive Behavioral Therapy (REBT) on the features of social anxiety manifested by university students.

2. METHOD

2.1. Participants.

A quasi-experimental ABA design was applied. A sample of 20 participants referred from counseling centre was recruited with inclusion criteria of a) 18-25 years of age b) depicting at least 3 out of 10 symptoms from DSM-V criteria. From there, a sample of 10 participants scoring above 19 on Social Phobia Inventory [20] was selected for the study. Since a focused grasp on beliefs and thoughts about oneself and corresponding social situations was essential for and Rational Emotive Behavior Therapy practices therefore, comorbidity with other medical or psychological condition including major depression, psychosis or any substance induced disorders were excluded.

2.2. Procedure.

A sample of 10 participants was selected by administering Social Phobia inventory for assessment. The participants were then randomly divided into two groups including experimental and control group. Intervention based on Ellis' rational-emotive-behavioral therapeutic model was implemented on experimental group consisting of; a) Cognitive therapy, e.g. identification of irrational beliefs and their disputing b) Emotional methods, e.g. sense of humor, modeling, and role playing c) Behavioral methods, involving skill training, use of reinforcement d) Home assignment. The therapy involved 8 sessions for 1 hour. It was held twice a week and therapeutic effects were traced after application of intervention. Post-test was applied on the participants from both groups via Social Phobia Inventory (SPIN). A follow-up of 2 sessions was also implemented.

2.3. Measure
2.3.1. Social Phobia Inventory (SPIN).
It is a 17-item self-rating scale which covers the symptoms of fear, avoidance and physical signs of social anxiety. These symptoms are basically the three important dimensions of Social Anxiety. The inclusion of four autonomic symptoms (trembling, blushing, and heart palpitations sweating) provides the practical enlightenment about the bothersome experience of symptoms in public. The measure is rated on a scale from 0 to 4. The total score for the SPIN ranges from 0 to 68. This scale depicted an acceptable test-retest reliability for the SPIN (r= 0.78-0.89) [20].

2.4. Ethical Considerations

A healthy endeavor regarding the ethical considerations was adopted. Permissions were sought from the counselor of the particular university. Permission to use the screening scale was also sought from the author. Informed consent from the participants was taken before implementing the therapeutic program. Discrimination on the basis of gender, race, religion etc was avoided. Precautions were adopted to minimize any possible harm and to maintain the element of privacy. A concise from Rational Emotive Behavior Therapy was used on the control group after the conduction of study. Beneficial steps to maximize the possible benefits were also taken.

2.5. Statistical Methods

Means and standard deviations were computed for Pre and Post test results. Non-parametric Statistics were applied due to sample size. Wilcoxon Signed Ranks Test was used for paired pre-post test comparison for both experimental and control groups. Kolmogorov-Smirnov Z two-sample test was used for the between group analysis.

3. RESULTS

3.1.   Demographic Characteristics.

Table 3.1 Demographic Characteristics of the Participants (N=10)

Category	REBT (n=5)			Control (n=5)		
	M(SD)	*f*	*%*	*M(SD)*	*f*	*%*
Age	19.40(1.14)			19.80(1.30)		
Gender						
Male		3	60.00		3	60.00
Female		2	40.00		2	40.00
Education						
Bachelors I year		1	20.00		1	20.00
Bachelors II year		2	40.00		1	20.00
Bachelors III year		1	20.00		1	20.00
Bachelors IV year		1	20.00		2	40.00

Note. f=Frequency, *%*=Percentage, *M*=Mean, *SD*=Standard Deviation, REBT=Rational Emotive Behavior Therapy

Table 3.1 illustrated that number of male were more than female students among groups. Moreover, participants from Bachelors IV year were greater in number within control group, whereas Bachelors II year showed more number of participants across Rational Emotive Behavior Therapy group.

Results for the main hypothesis regarding effectiveness of Rational Emotive Behavior Therapy among University students are mentioned in Table 2 and 3 below.

Table 3.2 Within Group Analysis for Rational Emotive Behavior Therapy and Control groups (N=10)

Category	Pre-Test (n=5)		Post-test (n=5)		Z-Score	p-value
	M	SD	M	SD		
REBT	27.20	7.36	22.80	3.70	-2.02	.04*
Control	27.60	4.82	31.20	2.16	-1.84	.06

Note. REBT group= Rational Emotive Behavior Therapy group, M=Mean. SD= Standard Deviation,*= p ≤.05

Table 3.3 Between Group Analysis for Rational Emotive Behavior Therapy and Control groups (N=10)

Category	n	Kolmogorov-Smirnov Z	p-value
REBT	5	1.58	.01
Control	5		

Note. REBT group= Rational Emotive Behavior Therapy group,*= p ≤.05

The results applied significant difference for Rational Emotive Behavior Therapy (M=22.80, SD= 3.70, p= .04) post-test suggesting a decline in the social anxiety symptoms from moderate to mild level of severity. Between groups analysis showed a significant pattern for Rational Emotive Behavior Therapy (Z = 1.58, p=.01) effectiveness as compared to Control group. These finding suggested that Rational Emotive Behavior Therapy was not only a meager treatment for the symptoms of social anxiety but an effective management procedure as well.

4. DISCUSSION

Results from the current study showcased an effectiveness of Rational Emotive Behavior Therapy as a decrease in the intensity of negative cognitive features of social anxiety was observed. An alleviation of severity related to the features of avoidance, physical symptoms alongside the fear in social anxiety was underpinned. These results suggest the effectiveness of Rational Emotive behavior Therapy on university students which was in line with the results of [21] and [22] who supported with the current findings which highlighted the efficacy of Rational-Emotive Behavior Therapy on reducing the symptoms of depression among the adolescent girls and male collegemen facing problems in performing regular exercise respectively.

For effective application of Rational Emotive Behavior Therapy ABC model of irrational beliefs was addressed based on the principle of the (A) adversity-occurrence of event and the reaction to that event (B) Belief-explanation of that event and its reaction (C) Consequences- emotions and behaviors after the occurrence of events [23]. The results from current study follow those principles in fashion of portraying that the individuals manifesting the features of social anxiety face main challenge of negative cognitions related to the experience of anxiety in so-

cial situations. This hypothesis was supported from the study conducted by [24] who presented that the socially anxious individuals manifest the core cognitive features of fear of negative evaluations by others, performance situations such as speaking, eating in public, engaging in conversations, or interacting with authority figures as observed from the results of the current findings. Current finding also revealed a marked pattern of behavioral disturbance which was supported by [25] who explained that behavioral problems in particular includes feelings of nervousness in the presence of people which may indulge individuals to behave in a certain way or say something and then feel embarrassed or after. Presence of physical symptoms while engaging in a social situation was also acknowledged from the current study. The findings were supported by [26] who highlighted the physical symptoms related to the anxiety experienced in social settings i.e. excessive sweating, blushing, palpitations, trembling, and nausea.

Findings obtained from the current study also reflect that social anxiety is most prevalent among the adolescents and young adults. These findings were correlated with the study conducted to find the prevalence of social anxiety among different populations. The study suggested that social anxiety often seemed to be experienced by adolescents or young adults, who are most of the time, avoids situations which may require performing tasks in front of people during their life [27].

4.1.   Limitations and Future Directions

Selection of a small sample size can be addressed in further researches to generalize the effectiveness of Rational Emotive behavior Therapy. In order to fully understand the domains of particular therapy in relevance to social anxiety symptoms, the implication of Rational Emotive Behavior Therapy intervention could be generalized to other populations. For completely understanding the impact of this approach on students' socially anxious symptoms, long term effects of the treatment and the extent to which the treatment has the impact could be essential for future researches. Moreover, replication of these results on student population is could validate the effectiveness of Rational Emotive Behavior Therapy.

5. Conclusion

Despite the aforementioned concerns and limitations, the present study that Rational Emotive Behavior Therapy was effective to decrease the social anxiety symptoms in University students and also plays a vital role in increasing their well-being. The three major domains of fear, avoidance and physical symptoms were all seemed to alleviate through the therapy.

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A New Third Order Iterative Integrator for Cauchy Problems

Muhammad Daud Kandhro[1], Asif Ali Shaikh[2], Sania Qureshi[3]

Department of Basic Sciences and Related Studies, Mehran University of Engineering and Technology,
Jamshoro, Pakistan

ABSTRACT

In this paper, an explicit and single step Runge-Kutta iterative integrator of third order has been developed which is used to solve both autonomous and non-autonomous type of initial value problems also called Cauchy problems in ordinary differential equations. Linear stability analysis with corresponding stability region is drawn and error analysis has been provided to confirm third order accuracy of the integrator. Inclusion of a partial derivative with respect to the dependent variable within two slopes of the integrator has improved its efficiency in terms of local and global truncation errors. Finally, numerical examples are provided to show performance of the proposed integrator in comparison with other existing methods having same order of local accuracy. The software MATLAB R2017b was employed in order to produce all the numerical results and graphical illustrations presented in this paper whereas the MATLAB code designed to get such numerical results for all the integrators under consideration have also been provided.

KEYWORDS: Runge-Kutta, Stability, Local Truncation Error, Non-autonomous, Cauchy problems.

1. INTRODUCTION

Ordinary differential equations usually express all natural phenomena we come across in this physical universe. Their usage is present everywhere in biology, environmental engineering, physical systems, business, and economics to name a few [1-3]. It is a common practice of physical and biological researchers around the world to study the mathematical modeling of various physical problems based upon Cauchy problems such as radioactive decay, population dynamics, mechanical systems, fluid flows, electrical networks, rate of chemical reactions and many more [4-6]. Many problems of mathematical physics can be represented in the form of such ordinary differential equations.

In many situations, the methods capable to obtain solution of certain mathematical models stand to be very hard and complicated and sometimes even fail to produce the required results [7-9]. There are various numerical methods to get approximate solution of an initial value problem in ordinary differential equations and this is because only one numerical method cannot serve the purpose to get the solution of every type of initial value problem. Specially, numerous nonlinear types of Cauchy problems have stimulated researchers to get either the new numerical methods or improve the existing ones [10-17]. Another reason is the computational effort and CPU time required by the methods to solve the problem. The authors in [18] have tried to reduce the number of slope evaluations per integration step for autonomous initial value problems whereas the authors in [19] have extended the research work to non-autonomous type of problems. Moreover, nonlinear iterative integrators are suitable for the initial value problems having singular solutions along the integration interval under consideration as discussed in [20-24].

We consider the general first order ordinary differential equation with an initial condition, also called Cauchy Problem, as given below:

$$\frac{dy}{dx} = f(x, y), \ y(x_0) = y_0 \tag{1}$$

Existence of unique solution of (1) is assumed for the integration interval of $x \in [x_0, x_n]$. Here, exact solution is denoted by $y(x_n)$ whereas the numerical solution is by y_n taking the step size $h = \dfrac{x_n - x_0}{N}$, where $N = 1, 2, 3, \ldots$

2. DERIVATION OF THE PROPOSED INTEGRATOR

The general form of a single-step explicit numerical integrator to solve an initial value problem is given as:

$$y_{n+1} = y_n + h\phi_f(x_n, y_n; h) \tag{2}$$

Corresponding Author: Sania Qureshi, Department of Basic Sciences and Related Studies, Mehran University of Engineering and Technology, Jamshoro, Pakistan. **Email:** sania.qureshi@faculty.muet.edu.pk

where $\phi_f\left(x_n,y_n;h\right)$ can be expressed in terms of Taylor series expansion of an arbitrary function $f(x,y)$ as follows:

$$\phi_f\left(x_n,y_n;h\right)=\sum_{p=0}^{\infty}\frac{h^p}{(p+1)!}\left(\frac{\partial}{\partial x}+f\frac{\partial}{\partial y}\right)^p f(x,y) \tag{3}$$

Further, the Taylor series expansion of $y\left(x_n+h\right)$ is of the form

$$y\left(x_n+h\right)=y\left(x_n\right)+hf+\frac{1}{2!}h^2\left(f_x+ff_y\right)+\frac{1}{3!}h^3\left(f_{xx}+2ff_{xy}+f^2f_{yy}+ff_y^2+f_xf_y\right)$$

$$+\frac{1}{4!}h^4\left(\begin{matrix}f_{xxx}+3ff_{xxy}+3f^2f_{xyy}+5ff_yf_{xy}+3f_xf_{xy}+f^3f_{yyy}\\+4f^2f_yf_{yy}+3ff_xf_{yy}+ff_y^3+f_xf_y^2+f_{xx}f_y\end{matrix}\right)+O\left(h^5\right) \tag{4}$$

The proposed integrator of the present article is of the form:

$$y_{n+1}=y_n+h\phi_{3stageRK}\left(x_n,y_n;h\right) \tag{5}$$

where

$$\phi_{3stageRK}\left(x_n,y_n;h\right)=b_1k_1+b_2k_2+b_3k_3$$
$$k_1=f\left(x_n,y_n\right),\ k_2=f\left(x_n+a_2h,y_n+hk_1\left(b_{21}+hc_{21}f_y\right)\right)$$
$$k_3=f\left(x_n+a_3h,y_n+h\left(b_{31}k_1+b_{32}k_2\right)+h^2c_{31}k_1f_y\right)$$

Expanding k_2 and k_3 in Taylor's series, we obtain

$$k_2=f+\left(ff_yb_{2,1}+f_xa_2\right)h+\left(\frac{1}{2}f_{y,y}f^2b_{2,1}^2+f_{x,y}fa_2b_{2,1}+ff_y^2c_{2,1}+\frac{1}{2}f_{x,x}a_2^2\right)h^2+$$

$$\left(\frac{1}{6}f_{y,y,y}f^3b_{2,1}^3+\frac{1}{2}f_{x,y,y}f^2a_2b_{2,1}^2+f_{y,y}f^2f_yb_{2,1}c_{2,1}+\frac{1}{2}f_{x,x,y}fa_2^2b_{2,1}+f_{x,y}ff_ya_2c_{2,1}+\frac{1}{6}f_{x,x,x}a_2^3\right)h^3+O\left(h^4\right)$$

$$k_3=f+\left(f_yfb_{32}+f_yfb_{31}+f_xa_3\right)h+\left(\begin{matrix}\frac{1}{2}f_{yy}f^2b_{32}^2+f_{yy}f^2b_{31}b_{32}+\frac{1}{2}f_{yy}f^2b_{31}^2+f_{xy}fa_3b_{32}+\\f_{xy}fa_3b_{31}+f_y^2fc_{31}+\frac{1}{2}f_{xx}a_3^2+f_y^2fb_{21}b_{32}+f_xf_ya_2b_{32}\end{matrix}\right)h^2$$

$$+\left(\begin{matrix}\frac{1}{2}f_{yyy}f^3b_{31}b_{32}^2+\frac{1}{2}f_{yyy}f^3b_{31}^2b_{32}+\frac{1}{2}f_{xyy}f^2a_3b_{32}^2+\frac{1}{2}f_{xyy}f^2a_3b_{31}^2+\frac{1}{2}f_{xxy}fa_3^2b_{32}+\frac{1}{2}f_{xxy}fa_3^2b_{31}+\\\frac{1}{2}f_yf^2f_{yy}b_{21}^2b_{32}+f_y^3fb_{32}c_{21}+f_yff_{xy}a_2b_{21}b_{32}+\frac{1}{2}f_yf_{xx}a_2^2b_{32}+\frac{1}{6}f_{yyy}f^3b_{32}^3+\frac{1}{6}f_{yyy}f^3b_{31}^3+\\f_{xyy}f^2a_3b_{31}b_{32}+f_{yy}f^2f_yb_{31}c_{31}+f_{xy}ff_ya_3c_{31}+f_yf^2f_{yy}b_{21}b_{31}b_{32}+f_xff_{yy}a_2b_{31}b_{32}+\frac{1}{6}f_{xxx}a_3^3+\\f_yf^2f_{yy}b_{21}b_{32}^2+f_xff_{yy}a_2b_{32}^2+f_yff_{xy}a_3b_{21}b_{32}+f_xf_{xy}a_2a_3b_{32}+f_{yy}f^2f_yb_{32}c_{31}\end{matrix}\right)h^3+O\left(h^4\right)$$

Substituting the result of k_1, k_2 and k_3 into (5) then equate the coefficients of powers of h up to h^3 with that of (4) to obtain the following order conditions:

$$b_1 + b_2 + b_3 = 1 \qquad a_2 b_3 b_{32} = \frac{1}{6} \qquad a_2 b_2 + a_3 b_3 = \frac{1}{2}$$

$$\frac{1}{2}\left(a_2^2 b_2 + a_3^2 b_3\right) = \frac{1}{6} \qquad b_2 b_{21} + b_3 b_{31} + b_3 b_{32} = \frac{1}{2} \qquad a_2 b_2 b_{21} + a_3 b_3 b_{31} + a_3 b_3 b_{32} = \frac{1}{3} \qquad (6)$$

$$b_2 c_{21} + b_3 c_{31} + b_3 b_{21} b_{32} = \frac{1}{6} \quad \frac{1}{2}\left(b_2 b_{21}^2 + b_3 b_{31}^2 + b_3 b_{32}^2\right) + b_3 b_{31} b_{32} = \frac{1}{6}$$

One of the solutions of the above nonlinear system (6) forms the proposed three-stage explicit RK iterative integrator of third order as given below:

$$k_1 = f\left(x_n, y_n\right)$$

$$k_2 = f\left(x_n + \frac{2}{3}h, y_n + \frac{2}{3}hk_1 + h^2 k_1 f_y\right)$$

$$k_3 = f\left(x_n + \frac{2}{3}h, y_n - \frac{1}{3}hk_1 + hk_2 - 2h^2 k_1 f_y\right) \qquad (7)$$

$$y_{n+1} = y_n + \frac{1}{4}h\left(k_1 + 2k_2 + k_3\right)$$

The above proposed iterative integrator (7) can be used to solve both autonomous and non-autonomous type of initial value problems in ordinary differential equations. After getting this new integrator, we will analyze it for its accuracy, convergence, order of consistency and linear stability. These are the important terms related to an iterative integrator for it to be acceptable in the field of computational and applied mathematics as proved in [25].

3. ERROR ANALYSIS

In order to obtain the local truncation error of the proposed integrator, a usual functional associated to the integrator has been considered, that is given below:

$$L\big(z(x), h\big) = z(x + h) - y_{n+1}$$

where $z(x)$ is an arbitrary function defined along the integration interval $[x_0, x_n]$ and differentiable as many times as required. Having expanded it into Taylor series about x and collecting the terms in h, the local truncation error under local assumption of the following form has been obtained that ensures at least third order accuracy of the proposed integrator:

$$T_{n+1} = \left(\begin{array}{l} -\dfrac{5}{24}f_y^3 f - \dfrac{1}{72}f_{x,x}f_y + \dfrac{1}{24}f_x f_y^2 + \dfrac{1}{72}f_{x,x,y}f + \dfrac{1}{216}f_{y,y,y}f^3 + \\[2mm] \dfrac{1}{72}f_{x,y,y}f^2 + \dfrac{1}{72}f_{x,y}f_x + \dfrac{1}{216}f_{x,x,x} - \dfrac{1}{72}f_{x,y}f_y f + \dfrac{1}{72}f_{y,y}f_x f \end{array}\right) h^4 + O\left(h^5\right) \qquad (8)$$

4. CONSISTENCY ANALYSIS

Definition 4.1 Given an initial value problem $y'(x) = f\left(x_n, y_n\right); \ y\left(x_0\right) = y_0$; an iterative integrator with an increment function $\Phi\left(x_n, y_n; h\right)$ is said to be consistent if

$$\lim_{h \to 0} \Phi\left(x_n, y_n; h\right) = f\left(x_n, y_n\right)$$

The increment function of the proposed integrator (7) is shown as:

$$\lim_{h\to 0}\Phi\left(x_n,y_n;h\right) \;=\; \frac{1}{4}\lim_{h\to 0}\left(k_1+2k_2+k_3\right)$$

$$= \frac{1}{4}\lim_{h\to 0}\left[\begin{array}{l} f\left(x_n,y_n\right)+2f\left(x_n+\dfrac{2}{3}h,y_n+\dfrac{2}{3}hk_1+h^2k_1f_y\right)+ \\[2mm] f\left(x_n+\dfrac{2}{3}h,y_n-\dfrac{1}{3}hk_1+hk_2-2h^2k_1f_y\right) \end{array}\right]$$

$$= f\left(x_n,y_n\right)$$

Thus, the proposed integrator is shown to be **consistent** with at least **third order accuracy**.

5. LINEAR STABILITY ANALYSIS

An iterative integrator should not produce entirely different results for very small changes in the input data, that is, it should be stable in order to be acceptable for use in solving practical problems in computational and applied mathematics. Numerical stability of an iterative integrator ensures the control of the magnitude of errors inherent to either the integrator or the initial value problem under consideration. Among various ways to check stability of the iterative integrators, we consider Dahlquist's test problem of the form

$$\frac{dy}{dx}=\lambda y(x);\;\; y(0)=y_0,\;\;\lambda\in C$$

Employing the proposed integrator (7) on this test problem, we obtain the following stability function whose linear stability region is shown by the unshaded region in the Figure 1.

$$k_1=\lambda y_n \;;\; k_2=\lambda y_n\left[1+\frac{2}{3}h\lambda+h^2\lambda^2\right];\; k_3=\lambda y_n\left[1+\frac{2}{3}h\lambda-\frac{4}{3}h^2\lambda^2+h^3\lambda^3\right]$$

Substituting all of these values in (7), the stability function is found to be of the form:

$$R(z)=1+z+\frac{1}{2}z^2+\frac{1}{6}z^3+\frac{1}{4}z^4 \text{ where } z=h\lambda.$$

Figure 1. The 2D stability region for the Proposed Iterative Integrator
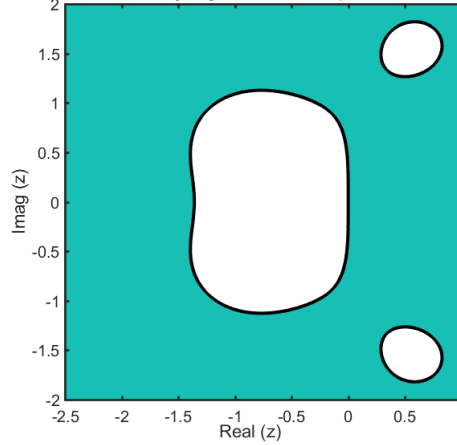
6. NUMERICAL EXPERIMENTS

In this section, some of the linear and nonlinear Cauchy problems in ordinary differential equations have been considered to show the behavior of the developed iterative integrator against other methods from well-established literature having same order of accuracy. Absolute maximum error, absolute error at the last nodal point of the given integration interval and CPU values for time have been presented to observe the performance of the developed method in comparison to other methods. Two standard methods called Runge-Kutta Method with Harmonic Mean of Three Quantities (RK3HM) [16] and Heun's third order method [25] as shown below have been chosen to compare the numerical results obtained through the newly developed iterative integrator.

Heun's Third Order

$$\begin{array}{c|ccc} 0 & 0 & 0 & 0 \\ 1/3 & 1/3 & 0 & 0 \\ 2/3 & 0 & 2/3 & 0 \\ \hline & 1/4 & 0 & 3/4 \end{array}$$

RK3HM Third Order

$$\begin{array}{c|ccc} 0 & 0 & 0 & 0 \\ 2/3 & 2/3 & 0 & 0 \\ 2/3 & -2/3 & 4/3 & 0 \\ \hline & k_1k_2/(k_1+k_2) & k_2k_3/(k_2+k_3) & 0 \end{array}$$

Table 1. Errors and CPU time values for Cauchy Problem 1

Problem 1. Nonlinear Cauchy Problem		
$\dfrac{dy}{dx} = xy^3 - y,\ \ y(0) = 1,\ \ \ y(x) = \dfrac{2}{\sqrt{2 + 4x + 2e^{2x}}}$		
Step-size/Method	RK3HM	Heun	Proposed
0.1	2.2406e-04	4.3314e-05	6.9364e-06
	2.1363e-04	4.0424e-05	3.8546e-06
	0.0000e+00	0.0000e+00	0.0000e+00
0.05	5.1698e-05	5.2797e-06	6.0548e-07
	4.9489e-05	4.9295e-06	1.4250e-07
	0.0000e+00	1.5625e-02	0.0000e+00
0.025	1.2497e-05	6.4999e-07	6.3439e-08
	1.1988e-05	6.0711e-07	7.4951e-10
	0.0000e+00	0.0000e+00	0.0000e+00
0.0125	3.0773e-06	8.0582e-08	7.2203e-09
	2.9553e-06	7.5283e-08	8.8340e-10
	1.5625e-02	1.5625e-02	1.5625e-02

Figure 2. Absolute Errors for Problem 1 with h = 0.1
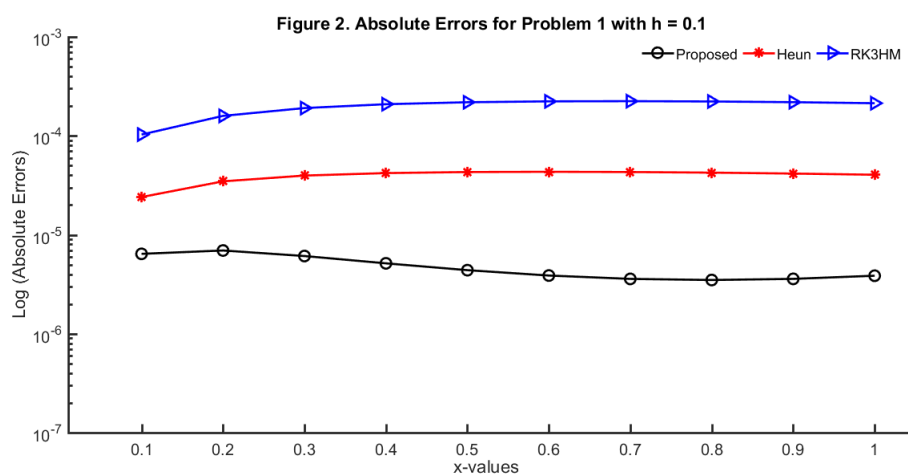
Table 2. Errors and CPU time values for Cauchy Problem 2

Problem2. Linear Cauchy Problem		
$\dfrac{dy}{dx} = x - y,\ \ \ \ \ y(0) = 1,\ \ \ y(x) = x + 2e^{-x} - 1$		
Step-size/Method	RK3HM	Heun	Proposed
0.1	2.0501e-03	3.3214e-05	3.7347e-05
	3.1498e-04	3.3214e-05	4.0107e-06
	0.0000e+00	0.0000e+00	0.0000e+00
0.05	6.1115e-04	3.9886e-06	4.1479e-06
	2.1978e-06	3.9886e-06	6.8968e-08
	0.0000e+00	0.0000e+00	0.0000e+00
0.025	1.9997e-04	4.8869e-07	4.8762e-07
	3.7473e-05	4.8869e-07	4.2089e-08
	0.0000e+00	0.0000e+00	0.0000e+00
0.0125	1.0845e-04	6.0478e-08	5.9073e-08
	5.3348e-05	6.0478e-08	7.2876e-09
	1.5625e-02	1.5625e-02	0.0000e+00

Figure 3. Absolute Errors for Problem 2 with h = 0.1
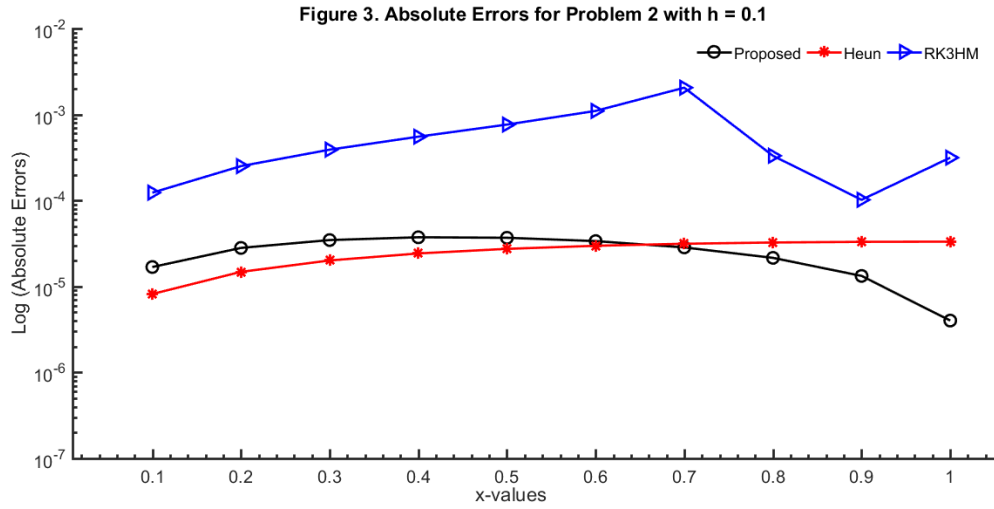
Table 3. Errors and CPU values for Cauchy Problem 3

Problem 3. Nonlinear Cauchy Problem			
$\dfrac{dy}{dx} = \dfrac{x^2}{y}, \quad y(0)=1, \quad y(x)=\sqrt{2\left(\dfrac{x^3}{3}+\dfrac{1}{2}\right)}$			
Step-size/Method	**RK3HM**	**Heun**	**Proposed**
0.1	1.5241e-03	8.3753e-06	3.5068e-06
	1.5241e-03	7.7843e-06	1.6030e-06
	0.0000e+00	0.0000e+00	0.0000e+00
0.05	3.8551e-04	1.0250e-06	3.6291e-07
	3.8551e-04	9.5083e-07	1.6466e-07
	0.0000e+00	0.0000e+00	0.0000e+00
0.025	9.7000e-05	1.2731e-07	4.5498e-08
	9.7000e-05	1.1762e-07	4.5498e-08
	0.0000e+00	0.0000e+00	0.0000e+00
0.0125	2.4332e-05	1.5843e-08	7.3060e-09
	2.4332e-05	1.4631e-08	7.3060e-09
	1.5625e-02	0.0000e+00	0.0000e+00

Figure 4. Absolute Errors for Problem 3 with h = 0.1
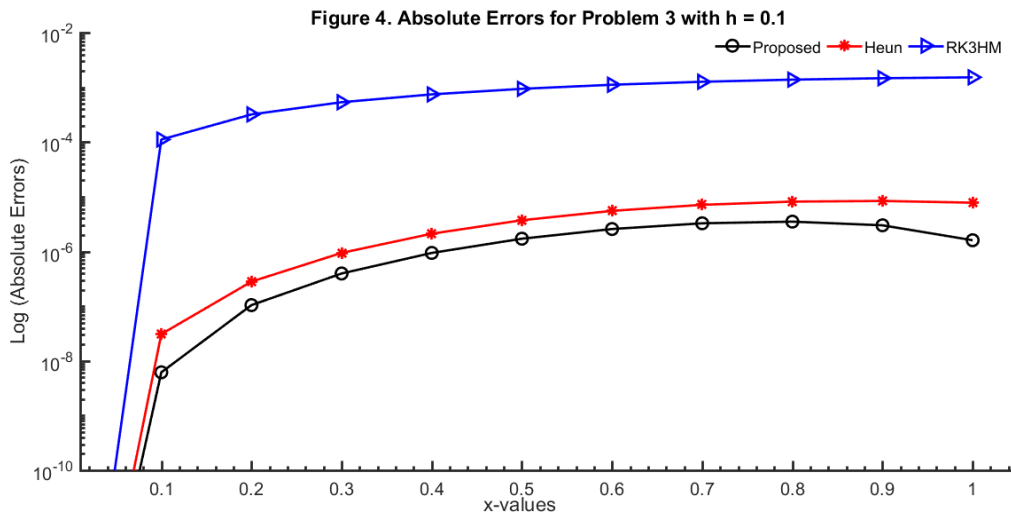
Table 4. Errors and CPU time values for Cauchy Problem 4

Problem 4. Linear Cauchy Problem		
$\dfrac{dy}{dx}=x^2y, \quad y(0)=1, \quad y(x)=e^{\frac{x^3}{3}}$		
Step-size/Method	**RK3HM**	**Heun**	**Proposed**
0.1	3.1171e-03	9.0292e-05	1.4165e-05
	3.1171e-03	9.0292e-05	1.3359e-05
	1.5625e-02	0.0000e+00	0.0000e+00
0.05	8.2411e-04	1.1713e-05	1.6414e-06
	8.2411e-04	1.1713e-05	1.2474e-06
	0.0000e+00	0.0000e+00	0.0000e+00
0.025	2.1194e-04	1.4897e-06	1.9677e-07
	2.1194e-04	1.4897e-06	1.2857e-07
	1.5625e-02	0.0000e+00	0.0000e+00
0.0125	5.3744e-05	1.8777e-07	2.4068e-08
	5.3744e-05	1.8777e-07	1.4347e-08
	0.0000e+00	1.5625e-02	1.5625e-02

Figure 5. Absolute Errors for Problem 4 with h = 0.1
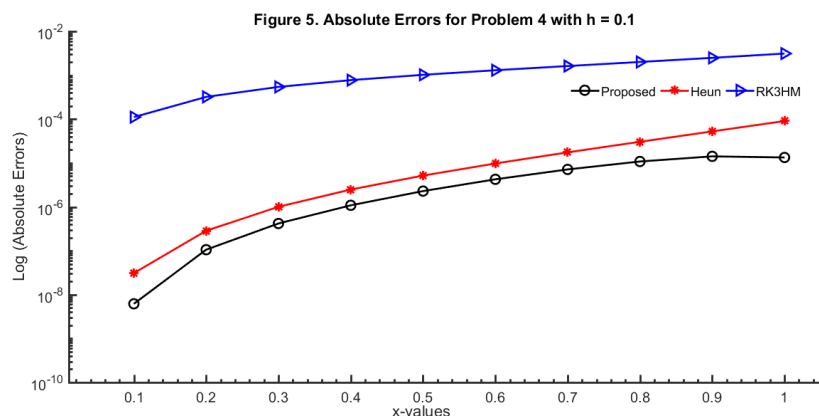
Problem 5. Nonlinear Cauchy Problem		
$\dfrac{dy}{dx}=xy^2-y, \quad y(0)=1, \quad y(x)=\dfrac{1}{x+1}$		
Step-size/Method	**RK3HM**	**Heun**	**Proposed**
0.1	3.9431e-04	3.6815e-05	1.4849e-05
	3.9431e-04	3.6815e-05	9.4940e-06
	0.0000e+00	0.0000e+00	0.0000e+00
0.05	9.2396e-05	4.4832e-06	1.5897e-06
	9.2396e-05	4.4832e-06	9.2296e-07
	0.0000e+00	0.0000e+00	0.0000e+00
0.025	2.2447e-05	5.5229e-07	1.8282e-07
	2.2447e-05	5.5229e-07	1.0023e-07
	0.0000e+00	1.5625e-02	0.0000e+00
0.0125	5.5374e-06	6.8510e-08	2.1895e-08
	5.5374e-06	6.8510e-08	1.1612e-08
	1.5625e-02	1.5625e-02	1.5625e-02

Figure 6. Absolute Errors for Problem 5 with h=0.1
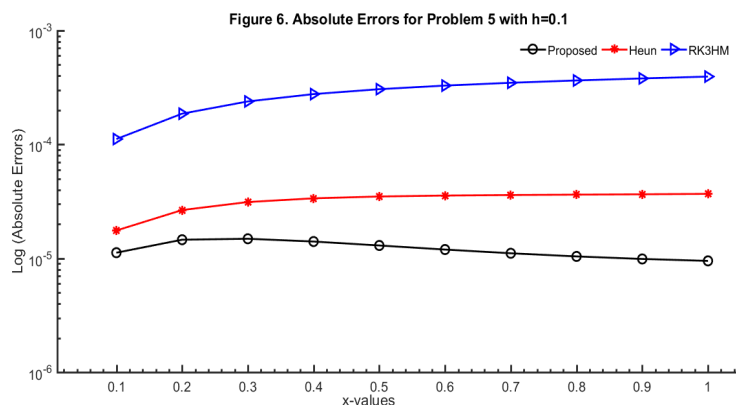
7. RESULTS AND DISCUSSIONS

The newly developed third order iterative integrator is capable of solving Cauchy problems in the field of computational and applied mathematics. The maximum error and last error with step sizes 0.1, 0.05, 0.025 and 0.0125 are tabulated along-with the values of CPU timing in seconds. One may observe from these tabulated data that the absolute maximum and last error produced by the proposed iterative integrator are much smaller than the errors produced by other methods having same order of accuracy while consuming same amount of time on average. Similar sort of behavior has been observed while taking the step-size as large as 0.1 as shown by the Figures 2-6 for all the iterative integrators under consideration. The numerical results obtained through the proposed iterative integrator produce numerical values approximately close to the exact solution in comparison to the values obtained through Runge-Kutta Method with Harmonic Mean of Three Quantities and Heun's third order method. For the proposed iterative integrator, small step size is also enough in comparison for other methods as shown in the Tables and the Figures above. Finally, it has been observed that the proposed iterative integrator is converging faster than the RK3HM and Heun's third order method and it is the most effective integrator for solving the Cauchy problems in ordinary differential equations as long as it is compared with the iterative integrators having same order of local accuracy as that of the proposed iterative integrator.

8. CONCLUSION

This paper develops a new single step Runge-Kutta iterative integrator for solving Cauchy problems in ordinary differential equations. The integrator is found to be third order accurate and explicit in nature. Its linear stability analysis gives the stability region which proves conditional stability of the proposed integrator. Examples in this paper proved that it is more accurate and effective integrator than some existing standard methods. Tables 1 to 5 above show the maximum error, the last error and CPU times related to all the integrators under consideration for the Cauchy problems with the variation in the step size. In addition, absolute errors produced by the above iterative integrators are smallest in case of the proposed integrator as shown by the Figures 2-6. The computations above evidently display the better accuracy of the integrator. The Runge-Kutta Method with Harmonic Mean grows faster in error than third order Heun and the proposed one. Hence, the proposed integrator performs best among the integrators taken for comparison. Based on the five Cauchy problems solved above, it follows that the proposed integrator is quite efficient specifically in terms of local accuracy. It can be concluded that the proposed integrator is powerful and effective in finding numerical solutions Cauchy type problems arising frequently in the field of computational and applied mathematics.

MATLAB CODE

```
% A New Third Order Iterative Integrator for Cauchy Problems
% Numerical Problem 1.y'=xy^3-y, y(0)=1;
% Exact Solution: y(x)=2/sqrt(2+4*x+2*exp(2*x));  Partial Derivative f_y=3*x*y^2-1
% Integration Interval [0,1]
clc; clear; close all; format shorte
x(1)=0; y(1)=1; h=0.1; xfinal=1; N=ceil((xfinal-x(1))/h);
Time_Proposed=cputime;
fori=1:N
    k1=f_der(x(i),y(i));
    k2=f_der(x(i)+(2*h/3),y(i)+(2*h/3)*k1+(h^2)*k1*(3*x(i)*y(i)^2-1));
    k3=f_der(x(i)+(2*h/3),y(i)-h*(k1/3-k2)-2*(h^2)*k1*(3*x(i)*y(i)^2-1));
y(i+1)=y(i)+(h/4)*(k1+2*k2+k3);
x(i+1)=x(i)+h;
end
Time_Daud=cputime-Time_Proposed;
t=x(1):h:xfinal;
Exact=2./sqrt(2+4*t+2*exp(2*t));
Error_Daud=abs(Exact-y);
Err_Max_Daud=max(Error_Daud);
Err_Last_Daud=abs(Exact(length(t))-y(length(t)));
semilogy(t,Error_Daud,'ko-'), hold on
%%
```

```
% Third Order Heun Method
TIME=cputime;
fori=1:N
    k1=f_der(x(i),y(i));
    k2=f_der(x(i)+h*(1/3),y(i)+(1/3)*h*k1);
    k3=f_der(x(i)+(2/3)*h,y(i)+(2/3)*h*k2);
y(i+1)=y(i)+h*(1/4)*(k1+3*k3);
x(i+1)=x(i)+h;
end
Time_Heun3=cputime-TIME;
Error_Heun3=abs(Exact-y);
Err_Max_Heun3=max(Error_Heun3);
Err_Last_Heun3=abs(Exact(length(x))-y(length(x)));
semilogy(t,Error_Heun3,'r*-')
%%
%3rd order MODIFIED RK Rule using Harmonic Mean
RK3HM=cputime;
fori=1:N
    k1=f_der(x(i),y(i));
    k2=f_der(x(i)+(2/3)*h,y(i)+(2/3)*h*k1);
    k3=f_der(x(i)+(2/3)*h,y(i)-h*(2/3)*k1+h*(4/3)*k2);
    y(i+1)=y(i)+h*((k1*k2)/(k1+k2)+(k2*k3)/(k2+k3));
x(i+1)=x(i)+h;
end
Time_RK3HM=cputime-RK3HM;
Error_RK3HM=abs(Exact-y);
Err_Max_RK3HM=max(Error_RK3HM);
Err_Last_RK3HM=abs(Exact(length(x))-y(length(x)));
semilogy(t,Error_RK3HM,'b>-')
%%
functiondydx=f_der(x,y)
dydx=x.*y.^3-y;
end
```

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In Silico Analysis to Predict Structure, Sequence Motif and Expression Level of Breast Cancer Biomarker Molecules: Protein and miRNA

Romana Siddique*, **Jannatul Ferdous**

Biotechnology Programme, Department of Mathematics and Natural Sciences,
BRAC University, 66 Mohakhali, Dhaka-1212, Bangladesh

ABSTRACT

Breast cancer is an important public health issue since it has become the reason of 69% cancer caused death in women throughout the whole world and 15% of cancer death in Bangladesh. According to recent findings, biomarker studies can help in the betterment of diagnosis, treatment and recurrence problem by constructing a biomarker panel. The project was designed to find the basic properties -structure, sequence motif and expression level of potential biomarker molecules of breast cancer. Only protein and miRNA were selected as biomarkers since they were easily collectable from body fluid. 11 proteins and 7 miRNAs were selected, as they were the one showing high specificity and sensitivity as biomarkers. The protein molecules were - ER, ER Beta, PR, TTR, Ki67, HSP60, Her2, CyclinD1, Cyclin E, P53 and CEA. The miRNAs were- miR10b, miR21, miR145, miR155, miR191, miR 382 and miR425. Bioinformatics approach was the fundamental base of this research to detect properties of these biomarkers. For structure SWISS MODEL Workspace (protein), mfold (miRNA), for sequence motif MEME, and to check expression level GEO Profiles were used. In the end of this study it was seen that CEA, TTR, ER, PR, ER Beta, Cyclin E and Ki67 were the proteins that could be a potential biomarker for breast cancer screening panel. CyclinD1, Her2, P53 along with miR155 are potential biomarkers for breast cancer staging and miR10b, miR21 can be potential biomarker for ER silencing treatment.
KEY WORDS: miRNA, cancer biomarker, P53, CEA, Cyclin E, Ki67

1. INTRODUCTION

Being a significant contributor to overall morbidity and mortality, breast cancer is by far the most frequent cancer among women in both developed and developing countries with an estimated 1.38 million new cancer cases (Kulasingam, 2008). And with 15% cancer death in women in Bangladesh (Sacha, 2014) and incidence rate 22.5 per 100000 in females (Rai, 2012). It has become a hidden burden in Banglqdesh. Studies have proved that, this condition is the result of the absence of suitable diagnostic or screening test for an early detection of clinically relevant breast cancer. The current screening methods used to detect breast tumors either benign or malignant, include clinical breast examination (CBE), mammography and ultrasound. (Kulasingam, 2008). All processes have their own limitations and also a very high false negetive rates (Kulasingam, 2008).

One of the most promising ways to achieve methods with improved sensitivity and specificity is through the use of cancer biomarkers. (Kulasingam, 2008). A joint venture on chemical safety, led by WHO with the United Nations and the International Labor Organization has defined a biomarker as "any substance, structure or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease."(Mandal, 2013.) Among all the different molecules in a human body- Protein and miRNA are known to perform the best as biomarker for specific diseases. Protein molecules are known as the best of biomarker molecules since they can easily be traced, studied and evaluated (Gam, 2010).

In the present study 11 protein molecules and seven miRNAs were selected (Table 1 & 2) to predict their structure, sequence motifs and expression level in cancer state. Protein molecules were :Estrogen Receptor (ER), Estrogen Receptor beta (ERbeta), Progesterone (PR), Transthyretin (TTR), Cyclin E, Cyclin D1, Her2(Human Epidermal Growth Receptor),Carcinoembryonic Antigen (CEA), P53, Heat Shock Protein60 (HSP60). When it comes to miRNA, Patterns of miRNA expression plays a very important role in oncogenesis. Because of their distinct patterns of expression associated with cancer type, remarkable stability in blood and other body fluids, miRNAs are considered to be highly promising cancer biomarkers (Zhao, 2010). Among the miRNAs, seven were selected for this study – miRNA10B, miRNA21, miRNA145, miRNA155, miRNA191, miRNA382, miRNA425.

Corresponding author: Romana Siddique, Biotechnology Programme, Department of Mathematics and Natural Sciences, BRAC University, 66 Mohakhali, Dhaka-1212, Bangladesh. Email: romanasiddique@gmail.com
Telephone:+880-2-8824051-4 Ext.4060 Fax:+880-2-58810383

The aim of this study was to explore the structure,motifs and expression level of some selected cancer biomarkers so that these can be used as a possible therapeutics in cancer treatments and for early brest cancer screening.

Table 1: Protein markers associated with Breast cancer

Protein	Accession number	Taxonomic name
ER	AAI28574.1	Estrogen receptor 1
PR	BAC06585.1	Progesterone receptor
HER 2	AAA75493.1	human epidermal growth factor receptor 2
CEA	CAE75559.1	carcinoembryonic antigen
KI67	NP_002408.3	Ki-67 protein
CYCLIN D1	AAH23620.1	Cyclin D1
CYCLIN E	NP_001229.1	G1/S-specific cyclin-E1
ER BETA	AAV31779.1	estrogen receptor 2 (ER beta)
TTR	CAG33189.1	Transthyretin
P53	BAC16799.1	Tumor protein p53
HSP60	AAF66640.1	heat shock protein HSP60

Table 2: miRNA markers associated with Breast cancer

miRNA	GI number	Taxonomic name
MIR10B	262206216	Homo sapiens microRNA 10b
MIR21	262205659	Homo sapiens microRNA 21
MIR145	262205329	Homo sapiens microRNA 145
MIR155	269846817	Homo sapiens microRNA 155
MIR191	262205347	Homo sapiens microRNA 191
MIR382	262206264	Homo sapiens microRNA 382
MIR425	262205357	Homo sapiens microRNA 425

2. METHODS

In the current study , all the sequences information for the miRNA were retrieved from miRBase (www.mirbase.org) and sequence information for proteins were retrieved from NCBI databse (https://www.ncbi.nlm.nih.gov/) and Uniprot(www.uniprot.org/) .In silico analysis for structure, expression level and sequence motifs were carried out for selected proteins and miRNAs.

2.1 Structure prediction
Mfold web server version 3.5 (unafold.rna.albany.edu/) was used to predict secondary folded structure of miRNA (Zucker M., 2003).
To predict protein structures, first the FASTA sequences were retrieved from a database, Uniprot (www.uniprot.org/). The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data. UniProt is a collaboration between the European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR)(www.uniprot.org/). Then blast was done using the Basic Local Alignment Search Tool (BLAST) (https://www.ncbi.nlm.nih.gov/BLAST/) with these sequences to find their best suited templates. After that alignment was checked with this sequence and their template with Clustal omega (https://www.ebi.ac.uk/Tools/msa/clustalo/**)**. And finally this alignment result was given as an input in the homology modelling website, Swiss model workspace (https://swissmodel.expasy.org/workspace/) which is a web-based integrated service dedicated to protein structure homology modelling. It assists and guides the user in building protein homology models at different levels of complexity (Arnold K, 2006).

2.2 Finding Sequence Motif
MEME Suite (meme-suite.org/) was used to discover sequence motif in both the cases of protein and miRNA molecules. The MEME Suite is a software toolkit with a unified web server interface that enables users to perform four types of motif analysis: motif discovery, motif–motif database searching, motif-sequence database searching and assignment of function. It offers a significantly expanded set of programs for these tasks compared with the earlier web server (Bailey, T. L., 2006).

2.3 Expression Level Observation
To find out the expression level of the selected proteins and miRNA molecules in different conditions of breast cancer, GEO (https://www.ncbi.nlm.nih.gov/geo/) of NCBI was used. GEO represents to Gene Expression Omnibus. The Gene Expression Omnibus (GEO) is an international public repository that archives and freely distributes microarray, next-generation sequencing, and other forms of high-throughput functional genomic data sets(Barrett T,2013 The GEO Profiles (www.ncbi.nlm.nih.gov/geoprofiles/) database stores gene expression profiles derived from curated GEO Datasets (https://www.ncbi.nlm.nih.gov/gds). Each Profile is presented as a chart that displays the expression level of one gene across all Samples within the Dataset.

3. RESULTS

3.1 Structure Prediction for miRNAs
Basically in this study, finding the secondary hairpin like structure of the miRNAs was the main focus. Knowing this kind of structure specifically is important because the folding in a certain way leads to the solution of different unanswered question in binding and functioning of that miRNA molecule. Secondary structure for seven miRNA moleculaes were predicted using Mfold (Fig.1)
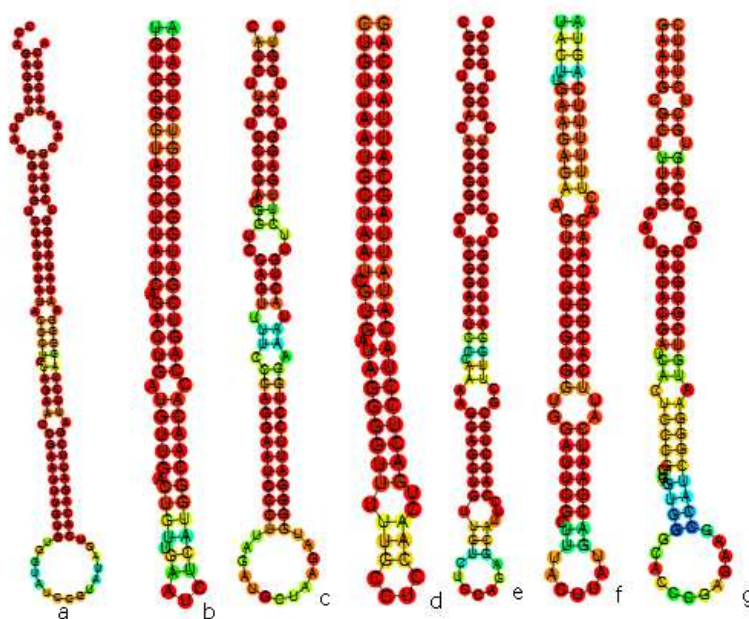
Fig 1: Hairpin structure of a) miR10B b) miR21 c) miR145 d) miR155 e) miR191 f) miR382 g) miR425

3.2 Motif Analysis for miRNA
For seven selected miRNAs, motif was observed (Fig.2) using MEME Suite (meme-suite.org/). Motif means a sequence that can have special importance biologically or functionally. Knowing motifs are important because they are recurrent and they indicate binding sites or functional sequence of that molecule.
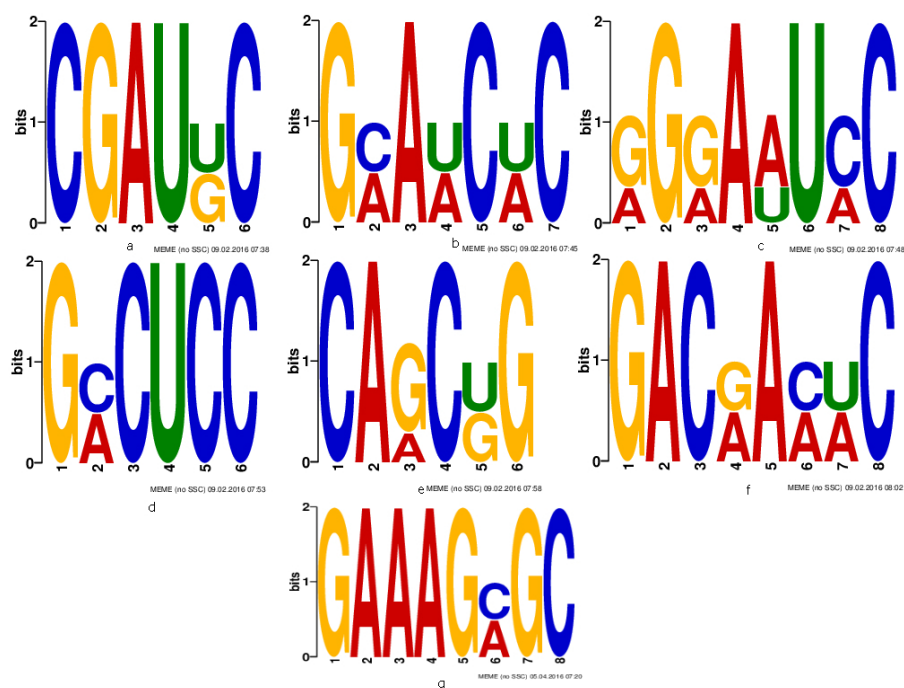
Fig 2: Important sequence motif of a) miR10B b) miR21 c) miR145 d) miR155 e) miR191 f) miR382 g) miR425

3.4 Analysis of Expression Level for miRNAs:

GEO tool was used to observe expression of seven miRNA molecules to observe expression pattern in different conditions of breast cancer. This is a tricky one to observe because this involves a multiple step to be expressed and also because this is an actual indicating property that makes the miRNA molecules a biomarker. Out of seven three miRNA molecule (miR10B, miR21, miR155) showed significant changes in their expression level. The expression level was measured in normal breast cancer patient and in patient whom were treated with ER mutation. It is seen that miR10B expression level is higher in the ER mutated cells and miR21 is low expressed in the ER mutated cells (Fig. 3).

This expression profile of miR155 showed expression in two different breast cancer cell lines along with a control. It was seen that in both cancerous cell lines (MDA-MB-436 and HCC 1954) miR155 is expressed differently than the normal cell line. It is expressed more in the MDA-MB-436 cell line and less in the HCC 1954 cell line (Fig. 3).
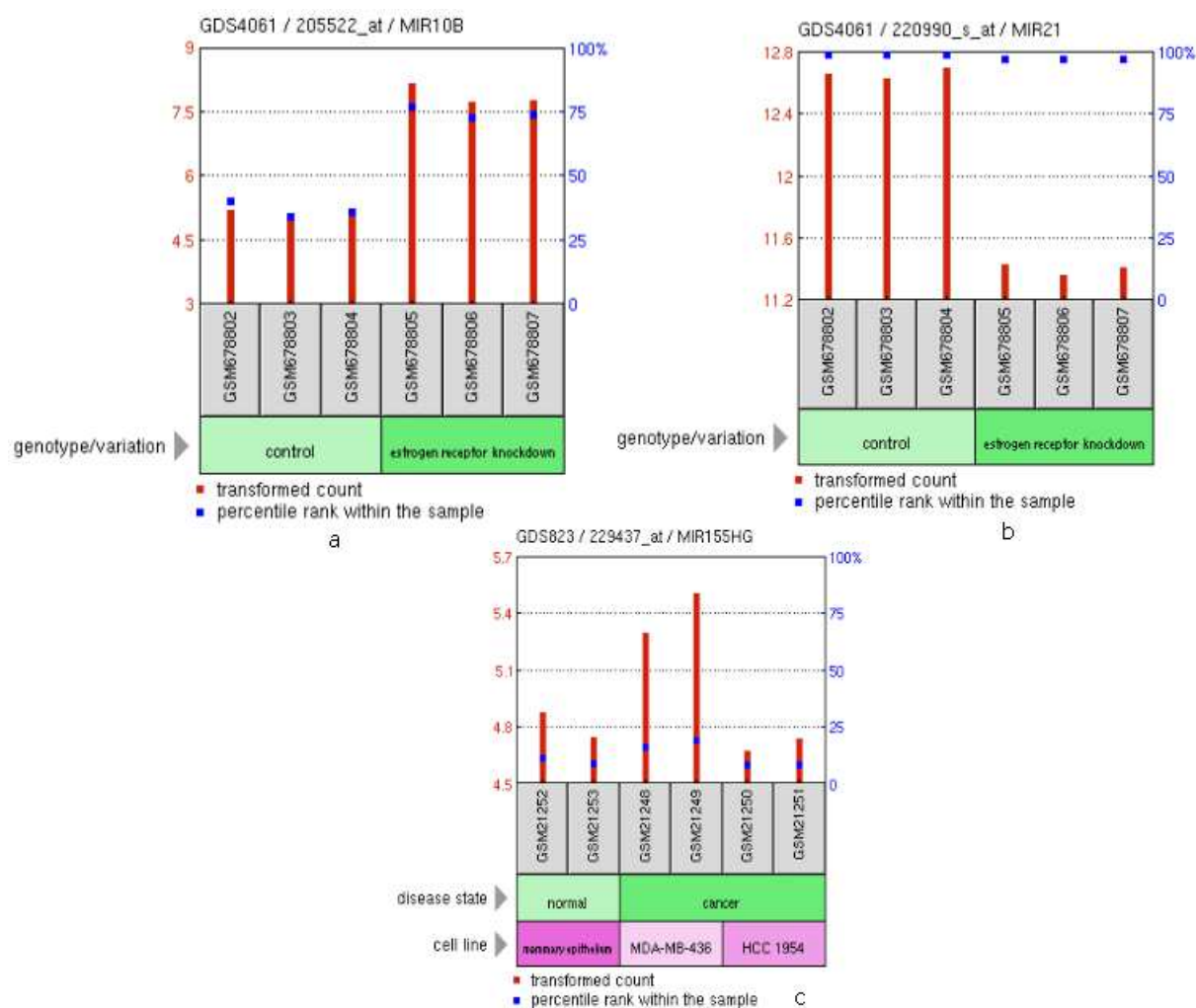
Fig 3: Expression level of a) miR10B b) miR21 c) miR155

3.5 Homology Modelling for Protein Biomarkers

Homology modelling was done using For the 11 protein biomarkers using Swiss model workspace (https://swissmodel.expasy.org/workspace/) (Fig.4). Predicting the homology model of these molecules can help in determining the structural motifs as well as site directed mutagenesis that might make them a candidate for biomarker panel of breast cancer.
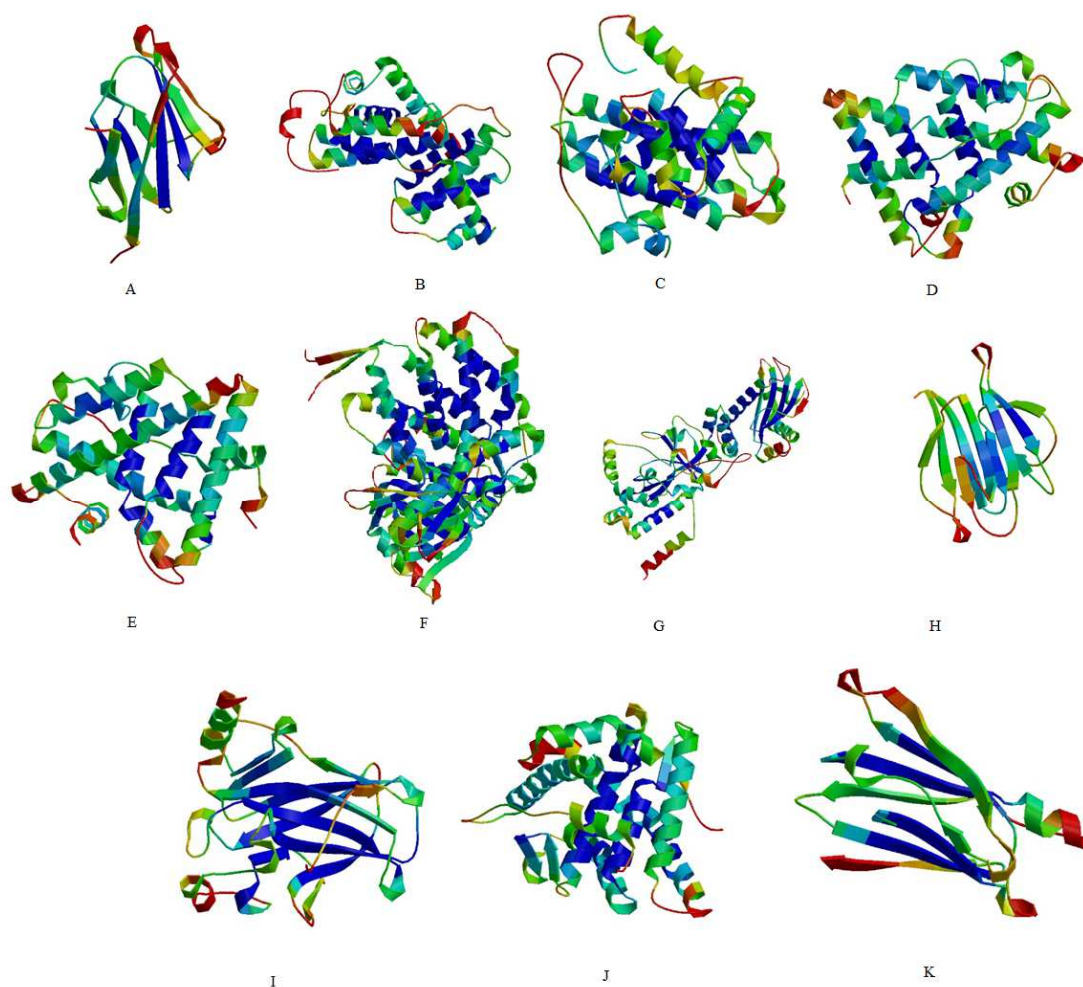
**Fig 4: Homology model of a) CEA b) Cyclin D1 c) CyclinE d) ER e) ER Beta f) HSP60
g) HER2 h) Ki67 i) P53 j) PR k) TTR**

3.6 MOTIF Analysis for protein biomarkers

Motifs for protein were also observed using MEME software (meme-suite.org/) was used and for each protein at least five motifs were observed. From these most significant motifs were selected (Fig.5). Knowing protein motifs are important because they give a clear information about the effects of sequence variation, protein interaction etc.
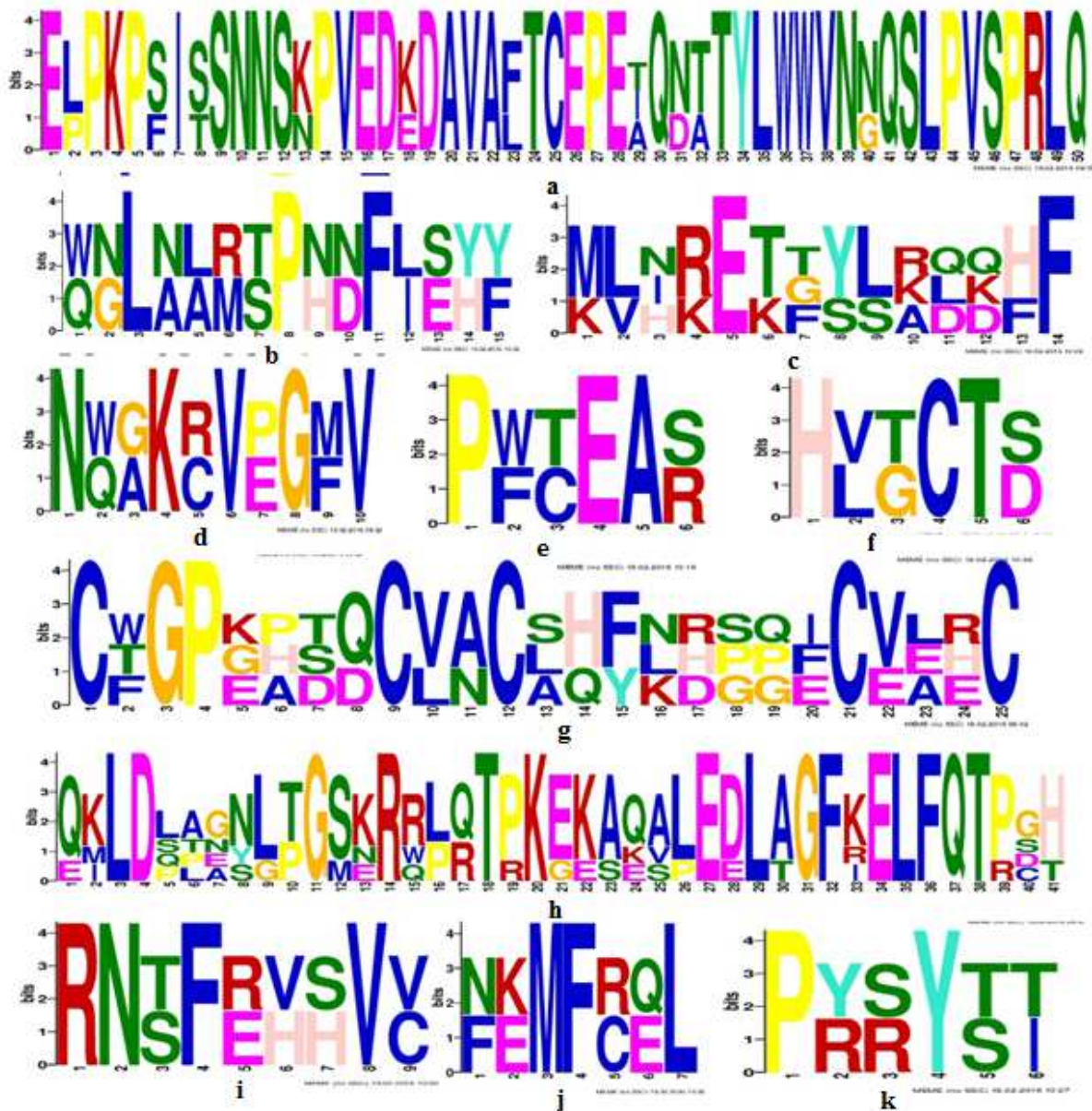
**Fig 5: Important Sequence Motif of a)CEA b)Cyclin D1 c) Cyclin E d) ER e) ER Beta
f) HSP60 g) HER2 h) Ki67 i) P53 j) PR k) TTR**

3.7 Analysis of Expression Level for Proteins
The expression of CEA, CyclinE, ER, ER Beta, HSP60, Ki67 and TTR were almost similar. All of them were over-expressed in both the breast cancer cell lines (MDB-MB-436 and HCC 1954) (Fig.6). On the other side expression level of Cyclin D1, Her2 and P53 were almost similar (Fig.6). They were more expressed in the HCC 1954 cell line and less expressed in the MDB- MB- 436 cell line. Lastly PR was differently expressed than all as it was under ex-pressed in both of the cell lines than the normal one.
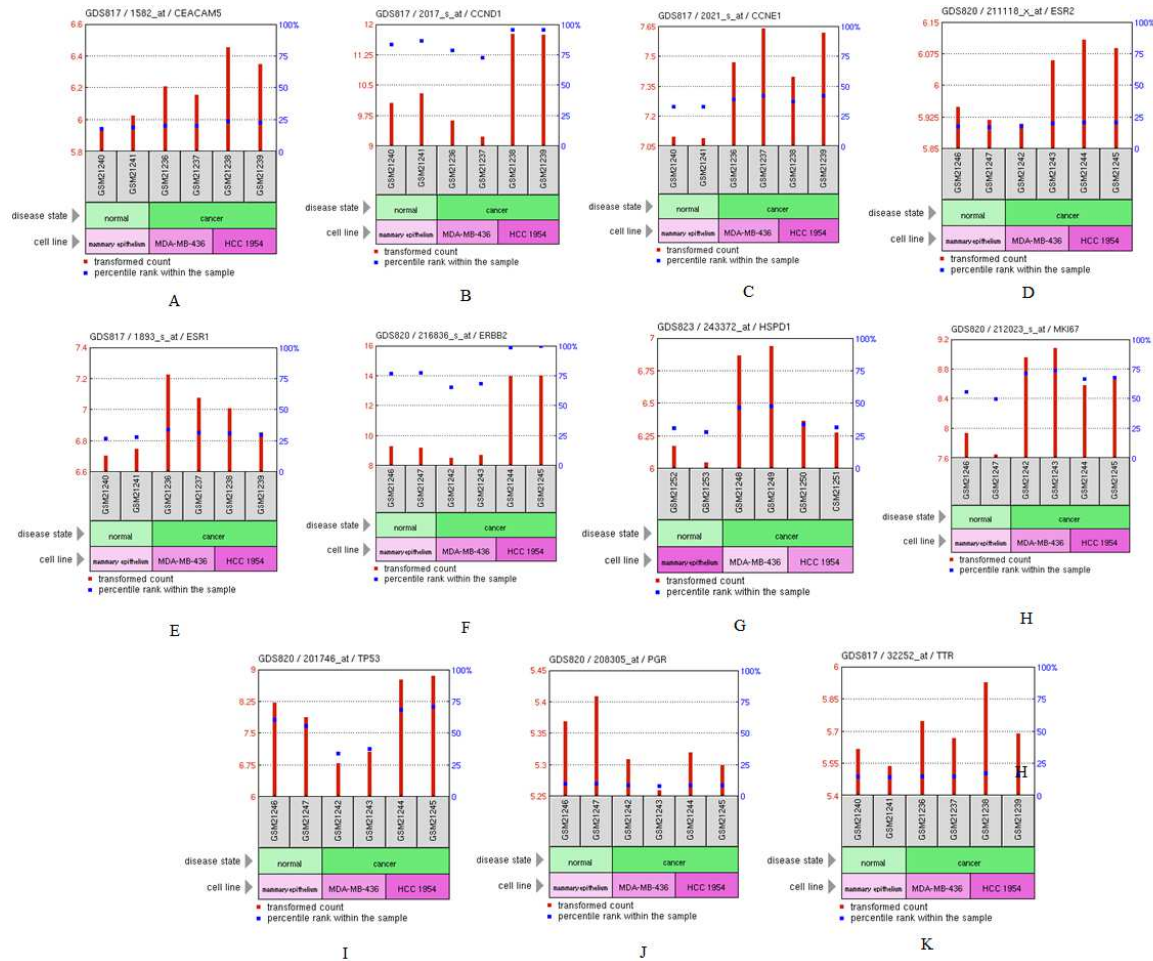
Fig 6: Expression level of a) CEA b) Cyclin D1 c) Cyclin E d) ER e) ER Beta f) HSP60 g) HER2 h) Ki67 i) P53 j) PR k) TTR

4. DISCUSSION

In the structure section secondary structure of miRNA and tertiary structure of proteins are shown. All the homology models are validated with QMEAN and the QMEAN score says all of them are more than 0.77% in a scale of 0 to 1.Sequence motif is the sequence that might have biological or functional importance. (Timothy, 1994).For each molecule maximum five motifs were commanded to be found and the standard length is from 6 to 50. Most of the motifs are present in two sites, but only a few are present in more than two sites. In the expression level observation results were taken from GEO Profile. From the huge store of different comparisons suitable results were taken. All these different cell lines are presented in the bottom light pink bars. And the diseased state are shown in the second bottom green bars. Above these two line of bars, ash colored bars show the name of the samples. In the long red lines that represents the transformed count of the expression level. This transformed count is from the actual experiment results as they were performed in affymetrix systems. And the blue squares presents their percentile rank among all the samples.

If these results are combined together a better biomarker panel could be decided with CEA, Cyclin E, ER, ER Beta, HSP60, KI67, TTR and PR. On the other hand Cyclin D1, Her2, P53 along with miR155can make a biomarker panel for breast cancer staging. mi10B and miR21 can play biomarker role in the ER silencing treatment systems.

5. CONCLUSION

Breast cancer is a global curse. This is the most commonly encountered cancer in our country as well as in the whole world (Y. Baskin, 2010). With an objective to add a little help in the findings of better treatment and diagnosis system this study was designed to know more about breast cancer biomarker molecules like protein and miRNA. It is hoped that individually the information of these biomarker molecule can help in finding a new therapeutic agent, site directed mutagenesis, virtual screening. Also together they can make a panel of biomarkers with better specificity and sensitivity that is needed the most at this moment. (Li, 2002).

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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EXPLORING PRACTICES OF NOVICE TEACHERS AT PRIMARY CLASSES IN PRIVATE SCHOOLS OF SKARDU, PAKISTAN

Ashiq Hussain[1], Dr. Muhammad Nisar Ul Haq[2], Aliya Kousar[3]

[1]Department of Education Development, Karakoram International University, Skardu Campus, Pakistan
[2]Assistant Professor, Department of Educational Development, Karakoram International University, Gilgit-Baltistan, Pakistan
[3]Department of Education Development, Karakoram International University, Skardu Campus, Pakistan

ABSTRACT

Novice teachers face different challenges as they endeavor to develop their teaching in new environment. In this study, we explored the practices of novice teachers which he/she used to overcome the challenges and how they learn through these practices. For this purpose, latest studies, challenges and practices of novice teachers were reviewed.In this paper researcher adopted both qualitative and quantitative approach as questionnaire used to collect data so it termed as quantitative whereas, descriptive statistics used to interprete and find out the results so qualitative technique also used in methodology. Researcher seeked answers to the questions from the real world.It gives deep undestanding in our context where crowded classes, non avalibility of resources and suitable learning environment have been overlapped.The researcher used semi structured interview guide consist of closed and open ended questions and a brief questionnair to collect the data.For quantitative data analysis SPSS is applied and for qualitative data analysis themitc approach and coding scheme are used.The major themes of this research paper are teaching and its aspect, School development and management, perception about novice teachers from senior teachers and experiences of novice teachers. Novice teachers consider that they are the builders of student's behavior, attitude and skills. They face many problems and tried to handle them. They counter problems in class controlling and handling, paper making and grading, adopting right methodologies and they strongly feel that they need workshops to overcome all above mentioned conditions.

KEY WORDS: Novice teachers, beginning teachers (BT), Mix Methodology, Private Schools, Gilgit-Baltistan

INTRODUCTION

The word "novice" has often been used in literature for new teachers, and as [6] noted, "there is no specific definition of a novice teacher in the literature. A novice teacher could be anyone who is teaching something new for the first time or who has entered a new cultural context for first time". There is also no compromise on how many years of teaching are essential to end this novice stage. Usually researchers define novice as "a teacher with less than five years of teaching experience"[14]. Teaching can be effective only if a teacher knows the personality traits of a child [15] and it would be more important in terms of novice teacher's techniques.Another important factor is "job satisfaction" in the teaching practices, if it gets combined with the organizational support and psychological well being [12].For this study, novice teacher was defined as a fresh Intermediate and Graduate teacher who has less than one year of teaching experience.

In current scenario, public sector schools are inducting experienced and professional teachers through proper test and interview system but comparing with private sector specifically in Gilgit-Baltistan, the teachers are inducting on their need based merely have some experiences in teaching. Teaching assessment through instructional practices can put a positive impact on the teaching practices [10]. At the same time, in-service training sessions can be helpful for empowering the teachers [23] and enhance the skills as well. Impact of experimental training sessions are also very helpful in enhancing the skills of the teachers [2].We can have a glance towards private schools especially primary level and we can find a large number of fresh intermediate and graduate teachers in this profession. How they are teaching? What kind of challenges they have to face while teaching? Are there any effects on learners due to these teachers? And can we provide any kind of favor to these novice teachers? Keeping all these facts in mind, the focus of this study is subterranean understand of challenges, practices, issues, problems, gender differences and learning style of novice teachers in the first year of their career as a teacher. The study mainly deals with the problem statement "Exploring practices of novice teachers at primary classes in private schools in Skardu, Pakistan".

*Corresponding Author: Dr. Muhammad Nisar Ul Haq, Assistant Professor, Department of Educational Development, Karakoram International University, Gilgit-Baltistan, Pakistan. Email: dr.nisar@kiu.edu.pk, Cell # +92-300-5553322

Objectives

1. To find out the problems /challenges faced by novice teachers during teaching.
2. To explore the ways of learning to teach.
3. To explore facilitating and hindering factors in learning to teach.

Research Question
What are the practices which novice teachers must adopt at primary level in Private schools of Skardu?

Problem Statement
To explore the practices of novice teachers at primary level in private schools of Skardu city. Due to disorganized scale system in private sector novice teachers cannot fulfill their duties in the atmosphere of satisfaction and empowerment. Practices are not well defined which can create a barrier in the operational measurements.

Significance of the study
This study is the unique study in education field specially in private schools where every year new teachers are inducted and this study helpful for the following :-
This study helps the novice teachers in their first teaching year's challenges like Classroom management, effective teaching techniques, understanding learner's level, and use full class room activities. It helps the principals of the schools to understand the challenges and effectiveness of novice teacher and also helps to deal with the novice teachers. This study helps the education lovers when they are going to discuss, giving presentation, delivering speech or they educating teachers through its reliable finding and facts and figure. It helps the administrators and owners of the institution to improve the performance of their institutions. As we know that in teaching and learning process the students are main components we do all these struggles for the better understanding and learning of students so through this study students are direct benefited. It helps the parents of the students to understand their challenges in learning and this study guides those parents how's children wants to become teacher.

LITERATURE REVIEW

The initial years of teaching are commonly considered by a "sink-or-swim" or "survival" mentality because we usually unable to present appropriate help for the development of teaching expertise [4]. When novice teachers want to teach they act like as experience teacher but actully they do not have the experienceto teach so they stuck themselves in many problems during the first year of their teaching. In the beginning years they must have opportunities for development [28]. However, the confrontation fluctuates in terms of person, institution and environment [22]. The term "novice" has often been used for new teachers, and as [6] noted, that there is no comprehensible definition for the term 'novice' but we can use this term to all those people who are teaching something newly. There is also no harmony on how many years of teaching are essential to end this novice stage.Some researchers defined novice teacher as a teacher who is related with the teaching field not more than five years [14]. Others passed on to the teacher who has teaching expeience not more than two years[9]. For the purpose of this study work, novice teacher is define as a teacher whose teaching experience is less than one year.When the figures concerning teachers' burnout rates are considered, it is not difficult to see how serious the situation is."25% to 50% of beginning teachers leave during their first three years of teaching, and nearly 10% leave in their first year" [16]. These all data point to the need to explore the issues and challenges of novices teachers faced and help them to overcome their problems in their beginning of teaching career. Novice teachers must have given basic teaching trainings [26]. In the context of Gilgit Baltistan, human resource in the educational projects recruitment and selection processes are negatively associated [3] where as, compensation, employee involvement and training & development are positively significant [3]. Satisfaction is a key factor which can secure the motivational factor within the job. A study inspects the role of job satisfaction and job stress on psychological wellbeing, turnover intentions and organizational support. The findings were according to the assumptions as perceived organizational support was positively corelatedwhile turnover intentions and job stress are inverse in relation [12]. Satisfaction can come by acquiring the skills within the atmosphere of jobthat is why a study shows the importance of in-service training can put positive impact on empowering the employees [23]. Experimental training is another tool to enhance he skills. A study shows that the effect of experimental training sessions of school teachers is positively significant on their performances [2].Education sector is enhancing according to "Pakistan Vision 2015". This can be done only if we prepare ourselves according to the the future requirements and face the challenges and get aware of the techniques how to confront the teachers' difficulties [1] because quality is the key requirement in terms of

attainment of futuristic goals. A study conducted at KPK shows that private secondary schools have positive contact with the development of KPK[17]. In 1999, Cornejo reflected on the issue and challenges of beginning teachers, taking as his focus the actuality of teachers in Latin America.In this conversation he pointed out the issue had been addressed in this region in minimal capacity; unlike, for example, what had been done in the United States and in some European countries.Thus, the adjoining reference we have on the matter is expressed in the contributions from those countries. Beginning teachers generally have to face different kinds of problems because they have to enter into a new type of environment for the first time in their lives. The study on support, identity and pedagogy provide a theme to evaluate the experiences of novice teachers and the challenges they have to face on their early stages [11]. [5] provides for classifying problems faced by BT in which four sections namely academic, social, organizational and technology are used from the semi structured plateform. Another big challenge for novice teachers is to find out the students psychological and inherent mental disorders. One of the genetic issues affected by some of the students is "Dyslexia" in which students feel difficulty in reading and writing. One of the study shows that mostly teachers have command on coaching the reading techniques but most of them are confused about dyslexia. So the certification and grades level do not properly calculate the authentic problem [27].Most of the work done on novice teachers learning by foreign researchers in their own context where they have all resources of teaching and learning available at schools. In Pakistan one of the researcher Dr. Haji Karim Khan [13] has made a qualitative study and interview sessions to one of the novice teachers of Primary school in the context of Karachi. He found out that novice teachers normally get frustrated in the preliminary days but gradually get use to it.In this paper researcher adopted both qualitative and quantitative approach for the study where researcher seeks answer to the question in the real world. It gives deep understanding about the crowded classes, non avalibility of resources and suitable learning environment.

METHODOLOGY

Researcher adopted Mix Methodology i.e, (both qualitative and quantitative approach) for the study. It is a study of particular novice teachers in a particular schools context of City Skardu (Gilgit-Baltistan) and it may not represent the experiences of all novice teachers' experiences in learning to teach in Pakistan. This study worked to explore the research question that what are the practices of novice teachers at primary level in private schools at Skardu? And how they can be effective according to the current scenario? Questionnaire is the useful way to collect data [20] so questionnaire and interview sessions are designed to collect the primary data for this study.

The population for the study included 56 private schools novice teachers (male & female) in Skardu city under an NGO named AFAQ. Eight Schools were taken as sample and selected two novice teachers from each school. 15 questionnaires are filled and interview sessions are conducted for novice teachers. The interviews support the quantitative data and gave better understanding about novice teachers' practices, their learning and overall school environment. Stratified random sampling technique is used and population has divided into four groups. Skardu main city, Gamba Skardu, Hussain Abad and Shaqthang area.

Two approaches 'thematic' and 'open coding' have used to analyze the data [8] "which involves reading though the data several times and then writing marginal notes to categorize the data into sections". Secondly, Researcher used SPSS which involves percentage representation in tables.

List of Schools

S.NO	Name of Schools
01	Public School and College Skardu Girls wing (Primary section)
02	Baltistan Higher Secondary School Skardu (Primary section)
03	New Children Public School Skardu (Primary section)
04	Al Hujjat Public School Shagari Kalan Skardu (Primary section)
05	Shaheen Public School Skardu (Primary section)
06	Ideal Scientific School Baltistan(ISSB)
07	Quaid-e-Azam Public School Skardu
08	Shining Star School Public School Skardu

DATA ANALYSIS

In this research study the sampling process is stratified random sampling where fifteen teaches (both males and females) participated from the eight private schools. The research participants were selected on the basis of their less

than one year professional experiences, so that the researcher can gather data according to the novice teachers' practices and their learning.

Table 1: Importance of Lesson Planning

		Frequency	Percent
Valid	**Strongly Agree**	13	86.7
	Agree	2	13.3
	Total	15	100.0

The above table shows 86.7% participants strongly agree and 13.3% agree that lesson planning is very important for teachers while there is no participant to disagree with the point.

Table 2: Teachers are changed agent

		Frequency	Percent
Valid	**Disagree**	2	13.3
	N/A	2	13.3
	Strongly Agree	3	20.0
	Agree	8	53.3
	Total	15	100.0

The above table shows that 53.3 % participants agree, 20% strongly agree, 13.3% disagree while 13.3 % neutral in their arguments.

Table 3: Co-curricular activities are mandatory part of education

		Frequency	Percent
Valid	**Strongly Agree**	8	53.3
	Agree	7	46.7
	Total	15	100.0

This table shows that all co-curricular activities are very important components of education and no Participant disagree with this point.

Table 4: Teacher is the developer of student's behavior

		Frequency	Percent
Valid	**Disagree**	1	6.7
	Strongly Agree	8	53.3
	Agree	6	40.0
	Total	15	100.0

The above table shows that all teachers agreed except one that teachers are the developers of student's behavior. It's a good sign that all new teachers know that they are the behavior developers of students but the challenge is to keep their efforts in a positive way.

Table 5: Whenever I feel any difficulty I shareit with other staff

		Frequency	Percent
Valid	**Disagree**	2	13.3
	Strongly Agree	7	46.7
	Agree	6	40.0
	Total	15	100.0

The above table shows that (40+46.7) 86.7% of teachers concern with other staff whenever they face any problem and this is the good learning attitude. While 13.33% do not share with other staff.

Table 6: Principal gives feedback on my teaching

		Frequency	Percent
Valid	**Strongly Agree**	6	40.0
	Agree	9	60.0
	Total	15	100.0

All Participants agreed that principal's feedback on their teaching illustrates strong commitment of head of the institution. In real sense the feedback of management gives the right way for the novice teachers' teaching. It is also a good quality of Principals that they provide feedback timely to their teachers.

Table 7: Classroom Management is very difficult task

		Frequency	Percent
Valid	Disagree	2	13.3
	N/A	1	6.7
	Strongly Agree	3	20.0
	Agree	9	60.0
	Total	15	100.0

Above table shows that 20% strongly agree and 60% agree that classroom management is very difficult task for them while 13% disagree and 6.7% not clear.

Table 8: I prepare my lesson at home

		Frequency	Percent
Valid	Disagree	1	6.7
	N/A	1	6.7
	Strongly Agree	9	60.0
	Agree	4	26.7
	Total	15	100.0

Preparation of lesson at home 60% strongly agree, 26.7% agree and 6.7% disagreed and 6.7% not clear. This shows that majority of participants prepare their lesson at home that is good sign but we need to know how they prepare and what they know about lesson planning. We will try to find out it in interview and in observation.

Table 9: I give feedback on students note book

		Frequency	Percent
Valid	Disagree	1	6.7
	Strongly Agree	9	60.0
	Agree	5	33.3
	Total	15	100.0

60% participants strongly agreed, 33.3% agree and 6.7% disagree against the statement of giving feedback on students note book. All participant are teaching in private schools and in private school they strongly follow few thing one of them is note book checking.

Table 10: Assigned task to students in group

		Frequency	Percent
Valid	Strongly Agree	8	53.3
	Agree	7	46.7
	Total	15	100.0

The above table shows that 53.3% strongly agree and 46.7% agree while no one against that they should assign tasks to students in group.

Table 11: As a teacher I design activities to enhance student's decision making ability

		Frequency	Percent
Valid	Disagree	1	6.7
	N/A	2	13.3
	Strongly Agree	6	40.0
	Agree	6	40.0
	Total	15	100.0

Against the above statement 40% strongly agree, 40% agree while 6.7% disagree and 13.3% not clear. This shows that participants design different activities to enhance student's decision making ability.

Table 12: I actively participate in school decision making

		Frequency	Percent
Valid	Disagree	2	13.3
	N/A	3	20.0
	Strongly Agree	2	13.3
	Agree	8	53.3
	Total	15	100.0

The above table shows that 13.3% strongly agree, 53.3% agree while 13.3% disagree and 20%neutral that they play role in school's decision making process.

Table 13: School provides us teaching resources

		Frequency	Percent
Valid	Strong Disagree	1	6.7
	Disagree	5	33.3
	Strongly Agree	5	33.3
	Agree	4	26.7
	Total	15	100.0

33.3% strongly agree, 26.7 % while 6.7 % strongly disagree, 33.3% disagree that school did not provide teaching resources. So cumulatively 60% in favor while 40% not in favor.

Table 14: I make question paper myself

		Frequency	Percent
Valid	Disagree	6	40.0
	N/A	1	6.7
	Strongly Agree	3	20.0
	Agree	5	33.3
	Total	15	100.0

The above table shows that 20% participants strongly agree, 33.3% agree while 40% disagree and 6.7 % mark not clear. It means that 53.3% prepare question papers while 46.7% do not make papers by themselves.

Table 15: I can tolerate the misbehavior of students

		Frequency	Percent
Valid	Disagree	2	13.3
	Strongly Agree	4	26.7
	Agree	9	60.0
	Total	15	100.0

Strongly agree 26.7% and 60% agree that they tolerate on misbehavior of students while 13.3% disagree that they do not tolerate the misbehavior of students.

Table 16: Students feel fear facing me

		Frequency	Percent
Valid	Strong Disagree	2	13.3
	Disagree	2	13.3
	Strongly Agree	2	13.3
	Agree	9	60.0
	Total	15	100.0

The table shows that 13.3% strongly agree and 60% agree while 13.3% strongly disagree and 13.3 % disagree about the statement of students feel fear facing them. Overall 73.3% articulate that students feel fear means students did not discuss their issues and problems with them so it may disturb the learning environment.

Table 17: Students discuss their problems with me

		Frequency	Percent
Valid	**Strongly Agree**	9	60.0
	Agree	6	40.0
	Total	15	100.0

The above table shows that 60% strongly agree and 40% agree while there is no participant who disagrees. It shows that all students discuss their problems with teachers. If we look towards the table 16 majority declare that students feel fear discussing their problems with the teachers.

Teachers' Interview
Researcher has collected data through a semi structured interview guide and then themes extraction out of the responds from participants against 14 items. The themes constructed are five in numbers. These are teaching and its aspects, school development and management, Role of principal, perception about themselves and senior teachers and experience of being a novice teacher.

To extract the themes researcher used coding scheme and the codes are given with complete descriptions. Through these seven items address the TA (Teaching and its aspects), one item addresses the SDM (School development and management), two items address RP (Role of Principal), one item address PS (Perception about themselves and senior teachers) and three items address Exp (Experience of being a novice teacher). Below is the analysis against these themes.

Construct No o1: Teaching and its aspects
"Teaching is the profession of Prophet" is the central theme of the participants and they consider it utmost honorable position. During the study some of the teachers revealed the terrified fact that they chose this profession because of the unavailability of other options and it is another fact that female teachers are more committed because it matches with their requirement, which reflects from their interpretation that they chose the profession because it suits them and their parents also feel comfort by giving their permission to their daughters in adopting teaching as a field.
New teachers seem committed with profession but they do not have the clear concept of teaching; some of them said its hard job and some were in the opinion that it is the process of transferring knowledge and modify students' concepts towards positivity.
When researcher inquires about the teaching methodologies then majority said that "we do not have any idea of method but we like [friendly] method". When researcher emphasizes to ask question about methodologies then some had understanding of activities or student-centered learning but the participant were unaware of applying such practices appropriately in classes. Whenever they feel any problem or difficulty they mostly take consideration of other teachers, coordinator and head. Private schools usually have sophisticated practices of first learn and then teach which can captivate students towards their teachers. When researcher asked about the problems of teachers usually face during the lectures in classes then majority of teachers replied with just about same replies. They demonstrated us a long list of problems including lack of AV aids, feeling intricacy to gather students' interest, slow learners, class controlling, course compilation etc.

Construct No 02: School Development and Management
The novice teachers' role in school development and management is not as much extensive as compare to senior teachers. When researcher inquires about their role, most of them said that management does not regard their ideas and suggestions.

Construct No 03: Role of Principal
When researcher asks from respondents about a chance given to them to head school then which steps they would initiate for the development of school? Answering to my question, one of the respondents said that it is near to impossible to attain such a chance but if it happens then firstly, I would change class timings and concentrate on time management. 9 out of 15 respondents amid that they will arrange/increase/provide all necessary teaching resources like (ECD resources, library, Classrooms, game kits, multimedia etc);rest of other 6 respondents wants to work for the betterment of school management and teacher's capacity building. Some of the respondents were also in favor of higher salaries for teachers because most of novice teachers are highly qualified and salary packages do not match with their qualifications. All the novice teachers shared useful ideas for the development of the school and learning capacity because they generally face all the ground deficiencies which usually management cannot observe. So novice teachers are the bank of ideas if management involves them in meeting and other activities they surely get unique and creative ideas.

Construct No 04: Perception about novice and senior teachers
Novice teachers feel uncomfortable during their first year of teaching because they start a new profession which is in fact more than a job. They have to serve than to work. They also face bundle of problems and they need supervision and guide in all aspects. The management supervision plays a key role to bridge the gap between novice and seniors teachers. During the interview 10 out of 15 respondents react that senior teachers help and guide them whenever they face any problem but 5 out of 15 respondents reply of negative behaviors from seniors. A point is also noted that generally management and head of schools also give preference to the ideas of senior teachers.

Construct No 05: Experiences of being a novice teacher
When it is inquired from the novice teachers that who inspire them in life? 10 out of 15 respondents answer that they are inspired from their teachers and they like their teaching methods. It means that a good teacher can modify a conception in a positive or negative manner so if they taught from a positive teacher then the positivity would definitely reflects in the behavior of the students and inspire them to be one like them.. Actually it is a teacher who reshapes the future of a student and it can be done by a devoted teacher. Rest of the novice teachers took name of their father, mother, brother or sister from whom they inspired.

Findings and Recommendations
Findings
The overall findings established after analyzing the data from the questionnaires and interviews are mentioned in points.
1. Novice teachers tried their best to perform without any professional qualification and experiences.
2. Novice teachers do not have clear idea about lesson planning, classroom management and teaching methodologies.
3. School management does not involve novice teachers in their decision making and also don't appreciate novice teacher's ideas.
4. Lacks of teaching resources create problems for novice teachers in implementation of teaching techniques.
5. Most of the novice teachers did not have the idea of holistic development so they just want to transfer the knowledge of information to the students.
6. Mostly novice teachers become the victim of inferiority complex because of the neglecting attitude from senior teachers usually in discussions, planning, gathering and miscellaneous activities.
7. Low salary in also a gigantic problem of the novice teachers as they work equally and provide their full time.
8. Students feel fear to face the novice teachers because normally attitude of novice teachers towards their students is rude(table 16)
9. Novice teachers try to develop themselves on practical grounds to face the issues and participate in the school activities which are necessary for an effective teaching but during the phase students get suffer.
10. Novice teachers admire the importance of classroom management but they are unaware of managing class in a particular order (table 07)
11. Novice teachers are familiar with the concept and significance of co-curricular activities (table 03)
12. Paper making is a difficult task it came to know that majority of novice teachers make paper themselves and they do not have any idea of learning domains (table 14)
13. Novice teacher knows that teachers are the developer of students' behavior but it is very difficult for new teachers to develop the behaviors of students.

RECOMMENDATIONS
On the basis of the findings of this study, some recommendations are prepared which are:

Recommendations for School Heads
Research work relevant some recommendation for the school head teachers. First of all head teachers must organize initially an orientation session for the novice teachers that at the beginning they get basic information about your school and teaching learning process in the school. Secondly, they must organized a teacher training workshop on some important topics like Lesson planning , assessment system, classroom management ,child psychology ,items writing and teaching methodologies or strategies etc. Thirdly, give them a friendly environment so that they can share their issues problems and ideas and appreciate their good work. Fourthly, in co-curricular activities give them responsibilities. Lastly , your organization only grow if you facilitate the teachers so provide some basic teaching resources to the teachers when teacher use these resources quality of education increases as well as image of you school are also become good.

My research also recommend that finance is the driving force for any person, so salary of teachers is also motivation factor for them if it will be increased and paid timely, it can give mental satisfaction to the teachers so they give their full concentration on teaching.

Recommendations for the novice teachers

As we know that when we hear something we usually forget but when we see something then we can remember most of contents and as says "Practice makes a man perfect" when we practically do something then we can better learn. So it is clear that practical work can enhance the personality of a person. Firstly, this study recommends reading some books which are available in market about teaching and attaining of workshops because now a day's many organizations are working for capacity building of teachers. Secondly, this is the era of information technology. Everyone has access to the internet and one must need to take benefit from the internet instead of spending lot of time on social media.

Recommendations for the Government Education department

As we all know that it is necessary for all private schools to register themselves from the education department and all schools are registered but there is no check and balance from the education department. In public sector professional teachers are inducted at secondary level but in private sector majority of teachers are fresh, usuallythey do not have any experience and professional degree. This study suggests for private school's management to provide refresher courses to their teachers by time to time and also evaluate the performance of the private school teachers especially novice teachers and provide feedback because private sector carry partial burden of the education and they have the half future of the nation in their hands.

Recommended capacity building course for new teachers

At the end, on the basis of research study here is the course outline for the novice teachers including most important topics. Following this course outline schools can easily build the capacity of their teachers in short period of time. It is also helpful for the organizations who are working for teachers, parents who wish their child become a teacher, it is a need analysis for educators and it helps the novice teachers to attain workshop or study on these topics to become a good teacher.

Day(s)	Topics	Duration
1st	Introduction to teaching	3 hours
2nd	Child psychology	3 hours
3rd	Annual planning (scheme of work)	3hours
4th	Lesson planning	3 hours
5th	Classroom Management	3 hours
6th	Teaching methodologies	3 hours
7th	Assessment (item witting)	3 hours
8th	Classroom observation	2 classes
9th	Classroom observation	2 classes
10th	Feedback on teaching	1 hours

Conclusion:

Skardu city is an emerging city of Gilgit-Baltistan where people are much continuous about the education of their children. In such scenario, to provide quality education especially at primary level is not more than a challenge. Government schools are running in their capacities but there is a myth among parents who usually show their concerns with the private schools looking towards the environment and learning opportunities more than the government schools. Novice teachers have become necessity of private schools as they have to run their operations in a smooth way. But due to less or no experience novice teachers frequently face different kinds of challenges and barriers during performing their tasks. Capacity building, in-job trainings, motivational measurements and providing empowerment are some optimistic factors but to standardize novice teachers according to need of the time issues regarding novice teachers should properly be addressed. Government should regulate check and balance system within the private sector and keenly undertaken the initial induction procedures (qualification, experience and skills). Their teaching methodologies, classroom management and motivational level should properly be analyzed and should be awarded according to their performance.

Research implications and future directions

- This study will help the novice teachers to overcome the challenges they faced while at the beginning of their teaching career.
- It will also help Principals and educationist as a guideline being a student of educational development.
- The results of the study will clear the directions in achieving futuristic goals.
- This study will motivate new educational researchers to come up with new creative and diversifying ideas.
- It will emphasize head of the institutions to optimistically treat novice teachers within their institutions.
- This study will also open new windows for novice teachers to play more skilled and motivated role in their capacities.

Future research has been directed for assessing teaching practices to detain in different private schools.

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Highly Heavy Metals Tolerant Fungi Isolated From the Sand of Polluted Beaches in the Area of Annaba - East of Algeria

**Ghania BOURZAMA[1,2] , Khedidja BOUDERDA[2], Abderrafik MEDDOUR[2] and
Boudjemaa SOUMATI[1,2]**

[1]Microbiology Laboratory, Department of Biochemistry, Badji Mokhtar Annaba University, Algeria
[2]Aquaculture & Pathology Research Laboratory, Badji Mokhtar Annaba University Algeria

ABSTRACT

Heavy metals are chemical compounds difficult to remediate through conventional technologies. The use of microorganisms such as fungi provides an alternative pathway for the removal of these pollutants from contaminated environments. For the first time in Algeria, this investigation concerns the isolation, from the sand of a polluted beach, of strains of metal tolerant fungi towards Copper, Lead and Zinc. The isolation was achieved on Potatoes Dextrose Agar, and the identification was followed by screening, tolerance index, minimum inhibitory concentration and assessment of the amount of uptake of each strain against the three heavy metals on Czapek Yeast Agar. The screening of the resistant fungal strains showed a high tolerance of *Aspergillus clavatus*, *Aspergillus terreus*, *Fusarium oxysporium*, *Penicillium chrysogenum* and *Trichoderma viride* to the three heavy metals. In some cases, the values of the minimum inhibitory concentration were up to 16000 mgl^{-1}. The amount of metal uptake was over expectation showing the highest uptake for Copper ions of 1.6 mgg^{-1} by *Aspergillus terreus*, and 3.3 mgg^{-1} for Lead ions by *Penicillium chrysogenum*. The greatest uptake for Zinc ions was 4.8 mgg^{-1} by *Penicillium chrysogenum*. The Tolerance Index showed a different statistical tolerance pattern of *Aspergillus clavatus* for Lead and *Aspergillus terreus* for Copper (P≤0.05). There was no statistical difference in the capacity to remove Lead by *Aspergillus* terreus, *Fusarium* oxysporium and *Penicillium chrysogenum* (P≤0.05).

KEYWORDS: Heavy metals; Pollution, Fungi, Tolerance Index; Uptake; Screening; Annaba; Algeria.

1. INTRODUCTION

If compared to water, heavy metals have a relatively high density [1]. They are classified as essential metals (Copper, Manganese, Zinc, and Iron) toxic at high levels, and nonessential metals (Cadmium, Lead, Mercury, and Nickel) [2]. In the environment, sources of heavy metals are natural or artificial processes through the anthropogenic impacts including discharges from the various industrial activities such as mining, smelting works, electroplating industry, as well as from the discharges of pesticides and phosphate fertilizers used in the agricultural sector [3,4,5]. Industrial wastewaters contain high concentrations of heavy metals which are transferred to animals and to human through the food chain [6]. Heavy metals are difficult to remediate because the natural environmental compartments (soil and water) are not able to totally eliminate these toxic elements [7]. The chemical treatment methods have many disadvantages including high costs, high energy and/or reagents requirements, and often lead to the formation of toxic complex products that necessitate waste products disposal. Restoring heavy metals through efficient and economical procedures requires the use of different complex options of metal-separating methods.

To some extent, the bioaccumulation of heavy metals by organisms, mainly microorganisms, can be successful [8]. A long-term exposure of microorganisms to high metal concentrations induces the development of resistance processes. Therefore, if isolated from contaminated wastewaters and soils with high concentrations of heavy metals, these microorganisms may represent a biological source for the removal of these metals [9].

In various ecosystems, the fungi can exhibit a rapid growth as well as the ability to uptake heavy metals [10]. This uptake of metals by the fungal biomass properties is divided into bioaccumulation (energy-dependent processes) and significant biosorption (binding of metals to the wall cell without energy) [11]. Fungi have also the ability to alter the chemical status of the metal ions through various processes such as

Corresponding Author: Khedidja BOUDERDA, Aquaculture & Pathology Research Laboratory, Badji Mokhtar Annaba University Algeria. gbourzama@yahoo.fr ; rafikmeddour@yahoo.ca

reduction, bioaccumulation, mobilization and immobilization [12]. They, therefore, can clean the environment, protects the biodiversity from the metals, and allow subsequent reuse [10]. It has been demonstrated that fungal strains, isolated from differently polluted areas, showed an important tolerance towards toxic metals and can provide a tool for the elimination heavy metal from polluted soils [9;13].

In Algeria, no attempts of isolation of heavy metals tolerant fungal strains were investigated. The present study is the first of its type in Algeria, and was conducted in order to isolate resistant fungal strains of five filamentous fungi; *Aspergillus clavatus*, *Aspergillus terreus*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Trichoderma viride* from a polluted beach in order to assess their tolerance to high concentrations of Copper, Lead and Zinc.

2. MATERIALS AND METHODS

2.1. Samplings
This investigation was carried out during three separated periods; December 2010, March and June 2011. Randomly, sand samples were collected from 3 stations located in two separated beaches in the littoral of Annaba city. Station 1 (**S1**) (Lat: 36° 51' 50" N ; Long: 7° 46' 43" E) and Station 2 (**S2**) (Lat: 36° 52' 30" N ; 7° 47' 57" E) are situated in the eastern part of Annaba city at Sidi Salem Beach which is directly under the influence of the urban sewage and the nearby industrial wastewaters and discharges of Fertial Fertilizers Complex. This beach also includes the estuaries of Meboudja and Seybouse wadis known as highly heavy metal contaminated outlets for the discharges of El Hadjar steel factory and the nearby industrial area [14]. Station 3 (**S3**) (Lat: 36° 57' 23" N ; Long : 7° 46' 50" E) is located in the northern part of Annaba city at Ain Achir beach which is fairly distant from the continental influence, but mostly under the Modified Atlantic Water current intrusion (**Fig.1**)

During the samplings operations, precautious measures were taken to avoid an eventual contamination from the surface of the sandy bottom. At each station, four slightly separated core samplings were carefully taken at a depth of -5 cm from the top of the bottom. For each station, the 4 core samples were added together in a sterilized glass bottle then transferred to the laboratory in a cool box at 4°C.
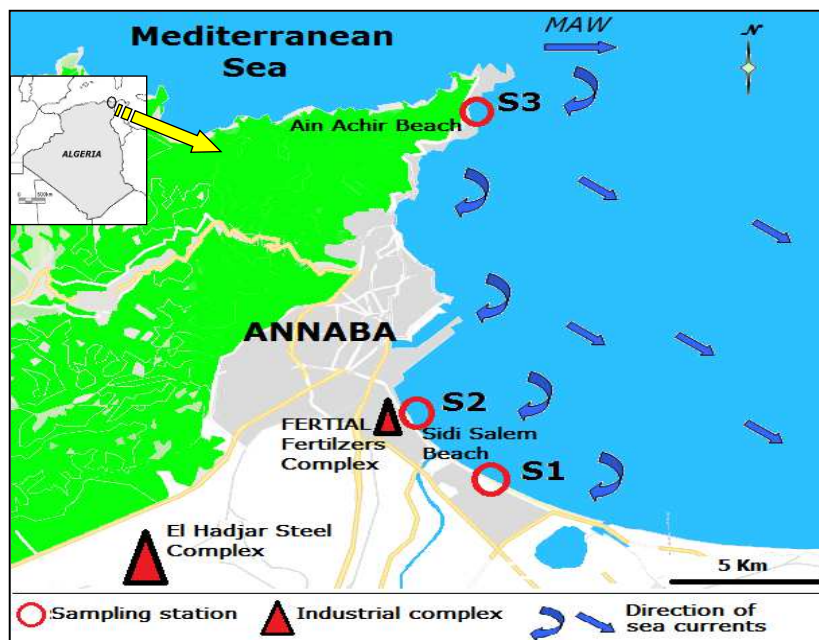

Figure 1: Sampling stations and main local sea currents
(MAW = Modified Atlantic Water current).

2.2. Heavy metals analysis of sand samples
Assessments of the concentrations of Copper, Lead and Zinc in the sand samples were carried out within the following 24 hours as indicated in the literature [15]. For each sample from each station, 1g of sand was mixed in a 50 ml flask with 10 ml from a solution of HNO3: HClO4 (1:2), then heated for half an

hour. After filtration through a Whatman filter paper (N°1), the volume was completed with distilled water up to 50 ml. The amounts of Cu, Pb and Zn in the digested sand samples were assessed by atomic absorption spectrophotometer as indicated in the literature [16]. The analysis was carried out in triplicate.

2.3. Isolation of strains
Within the 08 hours following the sand samplings, isolation of the fungi was made through the serial dilution method on Potato Dextrose Agar (PDA). The plates were incubated at 25±2°C for 7 days. The colonies were purified individually on PDA plates, and then incubated at 25±2°C for at least 7 days [17].

2.4. Identification of isolated fungus
All the fungal isolates on PDA were identified on the basis of the colony characteristics; the macroscopic (morphology, colour, shape and appearance of the colony) and the microscopic characteristics (septation and appearance of the mycelium, sporangiophore position, columella shape, diameter, texture of the conidia, and the spore shape). Various keys of identification were used [18,19,20].

2.5. Screening for heavy metals- tolerant fungi
Small agar plugs (5 mm) from young purified fungal of 7 days culture were screened for Copper (Cu^{2+}), Lead (Pb^{2+}) and Zinc (Zn^{2+}) tolerance in order to select the heavy metal tolerant strains. Concentrations of 200, 400, 600, 800 and 1000 mgl^{-1} of CuSO4, Pb(NO3)2, ZnSO4 were separately added to the CYA medium. The pH of the solid medium was adjusted to 6 with 1 M solution of Sodium Hydroxide before autoclaving [17]. The plates were incubated at 25±2 °C for 7 days. In parallel, cultures without heavy metals were carried out as a control bench [15,16].

2.6. Heavy metals Tolerance Index (TI) of fungi
The TI is an indicator of the response of an organism to a metal stress [21], taking into account that the higher the TI, the greater the resistance [22]. The TI was estimated as indicated in the literature [16]. For each plate on CYA and after 7 days of culture at 25±2°C, the diameter of the colony extension, from the point of inoculation, was measured for both colonies incubated in medium with heavy metals and colonies incubated in medium without metals (batch control). Measurements were carried out in triplicate. The mean value was respectively calculated for each strain and the TI was calculated as follows:

$$TI = \frac{\mathbf{D}t}{\mathbf{D}u}$$

With : $\mathbf{D}t$ = diameter of the radial extension (cm) of the treated colony
$\mathbf{D}u$ = radial extension (cm) of the untreated colony (control batch).

2.7. Determination of the Minimum Inhibitory Concentration (MIC)
The MIC of the isolates is defined as the lowest concentration of metals that inhibits the visible growth of the isolates. The three heavy metal ions were added separately to CYA medium at concentrations ranging from 200 mgl^{-1} up to the resistance level of 1600 mgl^{-1} with intervals of 200 mgl^{-1}. The metal ions added plates were inoculated with 5mm agar plugs from young fungal cultures of 7 days grown on a normal CYA medium. They were then incubated at 25±2°C for 7 days. The MIC of the fungi is considered after at least 7 days of growth [16, 22].

2.8. Removal of heavy metals by fungal isolates from liquid media
The tolerant fungal isolates were evaluated for uptake of heavy metals in a CYA broth medium containing concentrations varying from 200 mgl^{-1} to 1600 mgl^{-1} individually for Copper, Lead and Zinc. Flasks of 200 ml were inoculated with 5mm Agar plugs from young fungal cultures of 7 days, and then incubated on a shaker at 150 rpm, and at 25±2°C during 7 days. The Control batches of flasks without heavy metals were processed similarly.

After 7 days, the fungal growth was harvested. After centrifugation at 9000 rpm/s and filtration through a Whatman filter paper, each harvested fungal biomass was washed with double distilled water 2 to 3 times and dried in hot air oven at 70±2°C for 3 hours. The digestion of the dried fungal biomass was made with Nitric acid and Perchloric acid (3:1 ratio). The digested fungal biomass was filtered again through a Whatman filter paper and the volume was completed up to 50 ml in a volumetric flask. The heavy metal uptake was estimated using Atomic Absorption Spectroscopy (AAS) [23].

2.9. Statistical analysis

The difference in TI among the individual isolates was checked over in triplicate through statistical tests; one-way ANOVA and Post-Hoc multiple-comparisons (*Tukey test*) using the software SPSS 20. The difference is considered significant when $P < 0.05$.

3. RESULTS

.31. Heavy metals analysis of sand samples

All the measured data for the three heavy metals were compared with the standard limit values of the Act N° 220/2004 of the National Council of the Slovak Republic. The extremely high and above-limit values of Copper were found in the sand of the polluted beach at **S1**, while Lead and Zinc were moderate at **S2**. Copper was found as the main pollutant at **S1**. On the other hand, the limit values for Lead and Zinc were exceedingly high at **S2**, whereas the value of Lead was higher than those of Copper and Zinc at **S3** (**Tab.1**).

Table 1: Heavy metal contents in the sand samples (mg/kg).

Sampling Stations	Copper	Lead	Zinc
S1	1.2	0.99	0.4
S2	94.5	68.4	7.8
S3	0.18	0.31	0.09

3.2. Isolation of strains

25 species belonging to 16 genera were isolated from S2 and S3. Besides, 38 species belonging to 19 genera were isolated from S1 that exhibited the highest fungal diversity. *Aspergillus clavatus* was only found in the sand of the S1.

3.3. Screening for heavy metals tolerant fungi

Most of the isolates showed a high resistance against one metal at least. Isolates of *Aspergillus clavatus*, *Aspergillus terreus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, and *Trichoderma viride* showed a high resistance against Copper, Lead and Zinc at a concentration of 1000 mgl^{-1}, with a clear difference in their degree of growth in the presence of metals.

3.4. Heavy metals Tolerance Index of fungi

Investigated at various concentrations of Cu^{2+}, Pb^{2+} and Zn^{2+}, the TI of *Aspergillus clavatus*, *Aspergillus terreus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, and *Trichoderma viride* revealed different patterns for each metal. Copper and Zinc were tolerated by *A.terreus*, *A.clavatus*, *F.oxysporium*, *T.viride*, and *P.chrysogenum*, while Lead is only tolerated by *A.clavatus*, *A.terreus*, *T.viride*, and *F.oxysporium* (**Fig.2**).

The results from the mean values (n=3) demonstrated that the TI showed different statistical tolerance patterns of *A.clavatus* for Lead and *A.terreus* for Copper ($P \leq 0.05$). On the other hand, there was no statistical difference in the capacity to remove Lead for *A.terreus*, *F.oxysporium* and *P.chrysogenum* ($P < 0.05$).
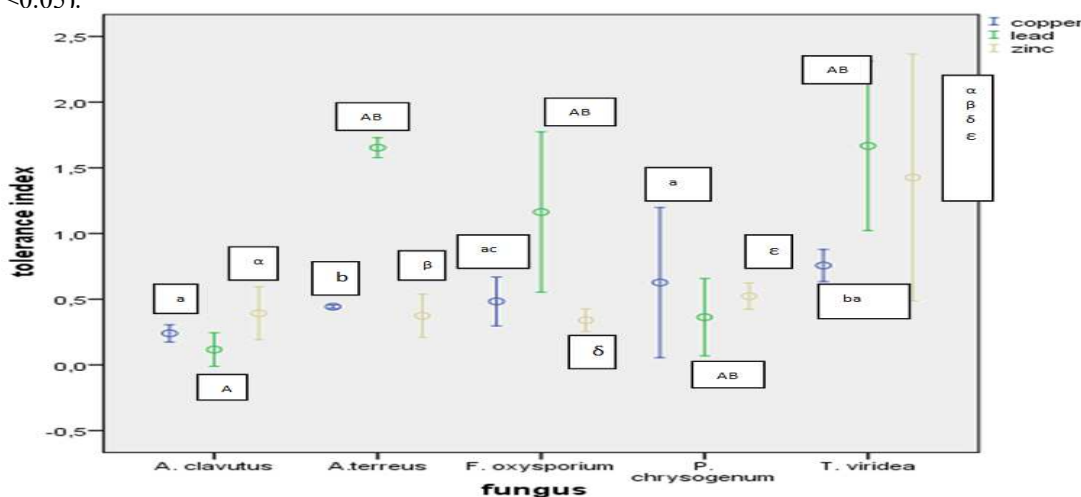

Figure 2: Tolerance Index of the fungal strains at different concentration of Copper, Lead, Zinc ions on CYA at 25 °C after 7 days. The different letters show the significant difference ($P \leq 0.05$)

3.5. Determination of the Minimum Inhibitory Concentration (MIC)

Depending on the fungal isolates, the data of the MIC revealed various resistance levels towards the heavy metals:

- *A.terreus* and *P.chrysogenum* strains showed more resistance against Copper with a MIC of 1600 mgl^{-1} higher than all other tested fungal strains (**Fig.3**).

- *A.terreus* and *T.viride* were the most Lead resistant strains with a MIC up to 1600 mgl^{-1}. *T.viride* and *F.oxysporum* exhibited a better growth at 400 mgl^{-1} than at 200 mgl^{-1} (**Fig.4**).

- *A.terreus* and *P.chrysogenum* tolerated Zinc concentrations up to 1600 mgl-1 (**Fig.5**).

- All fungal strains exhibited a better growth at lower concentrations of heavy metals but it became reduced in the presence of higher concentrations excepting for *P.chrysogenum* and *T.viride* against Copper.
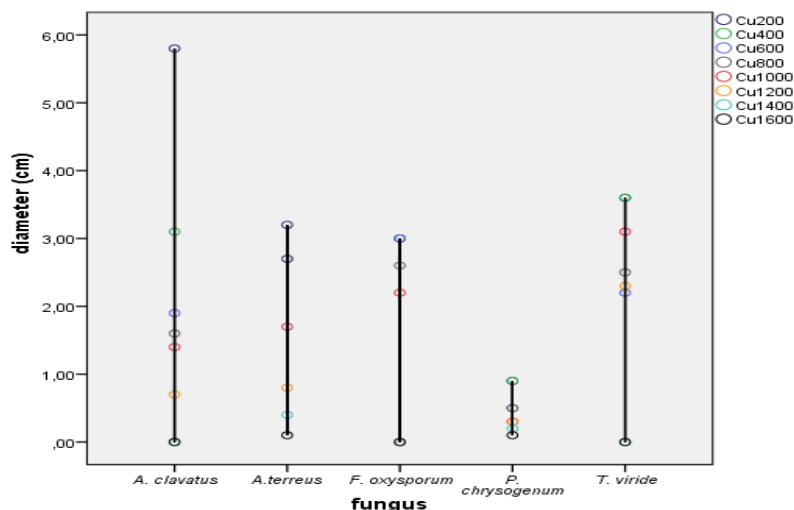

Figure 3: MIC of the fungal strains at different concentration of Copper ions on CYA at 25 °C for 7 days.
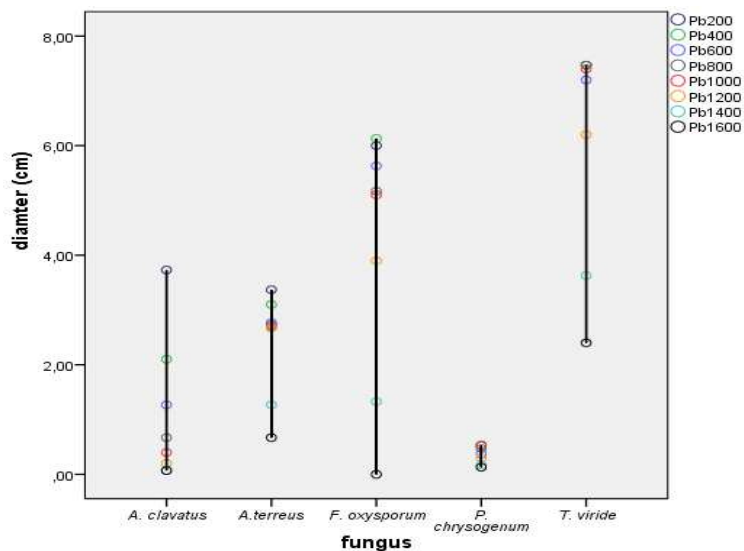

Figure 4: MIC of the fungal strains at different concentration of Lead ions on CYA at 25 °C for 7 days.
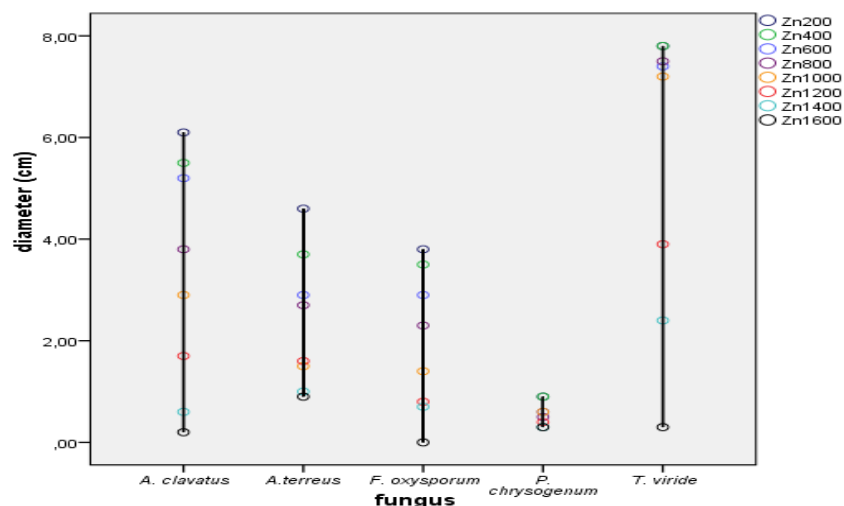
Figure 5: MIC of the fungal strains at different concentration
of Zinc ions on CYA at 25 °C for 7 days.

3.6. Heavy metal Uptake by the fungal strain

The highest recorded uptake of heavy metals were 1.6 mgg^{-1} of Cu^{2+} for *A.terreus* at 400 mgl^{-1}, 3.3 mgg^{-1} of Pb^{2+} for *P.chrysogenum* at 800 mgl^{-1}, and 4.8 mgg^{-1} for Zn^{2+} for *P.chrysogenum* at 1200 mgl^{-1}. It was also noticed that the heavy metal accumulation increased as the amendment concentrations increased until reaching the highest levels of accumulation. For all the tested strains, the uptake amount of 1600 mgl^{-1} was recorded for the three heavy metals.

Over time, the removal of Lead showed a similar profile to that for Zinc, except that the absolute amount of lead removed was in an order of magnitude less than for zinc. The amount of Cu^{2+}, Pb^{2+} and Zn^{2+} removed (absorbed and adsorbed) increased in response to the augmented heavy metals concentrations (**Fig.6,7,8**).
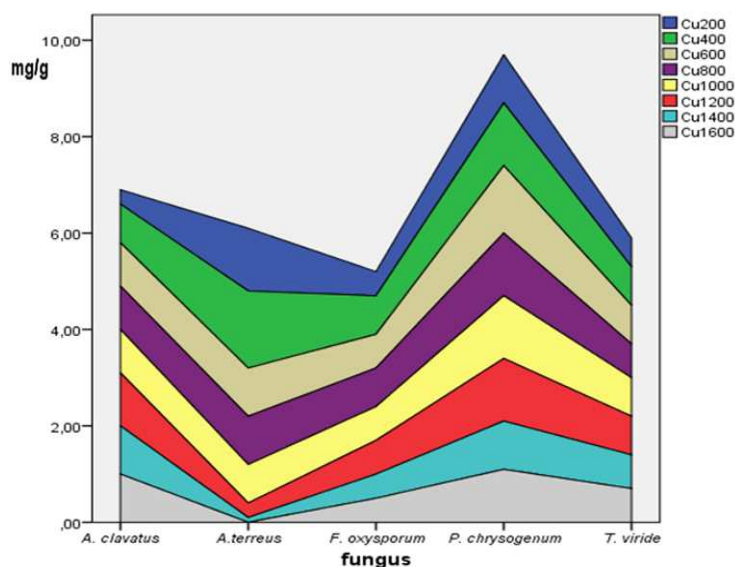

Figure 6: Uptake amount of Copper ions by the five fungal strains on CYA at 25 °C for 7 days.
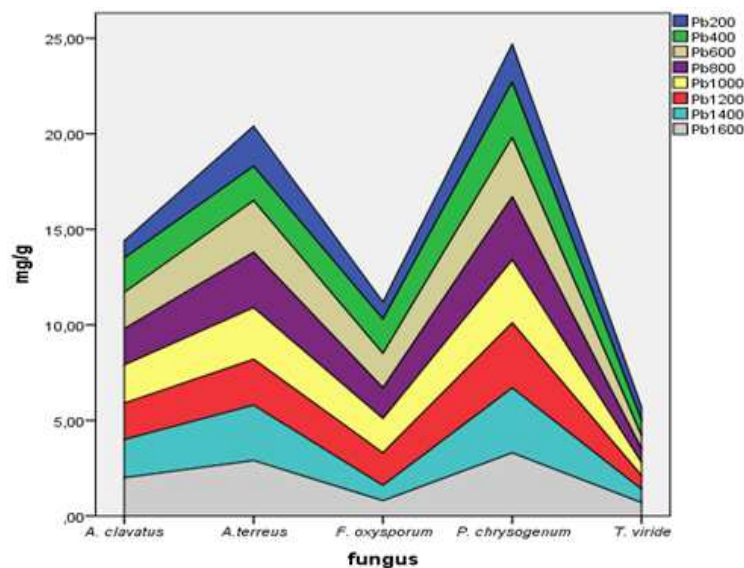
Figure 7: Uptake amount of Lead ions by the five fungal strains on CYA at 25 °C during 7 days.
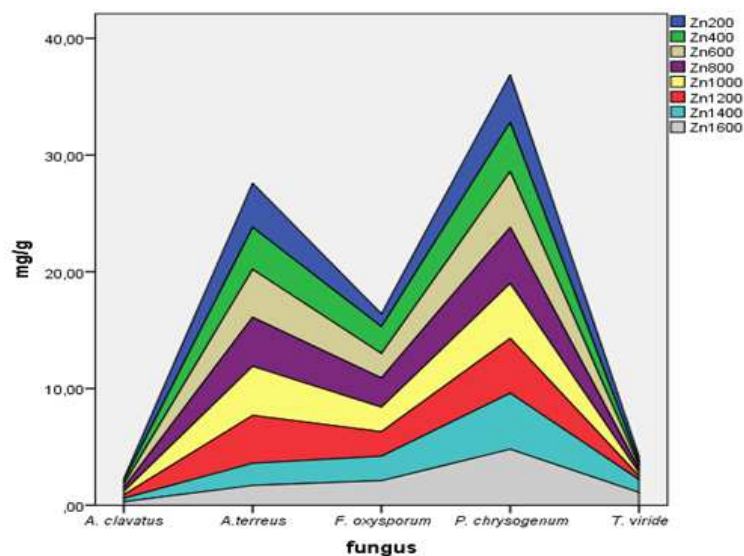

Figure 8: Uptake amount of Zinc ions by the five fungal strains on CYA at 25°C during 7 days.

4. DISCUSSION

The results of the sand analysis suggest that the two investigated areas, Sidi Salem Beach and Ain Achir beach are contaminated by the pollutant flows from the local urban and industrial wastewaters and discharges despite the fact that **S1** is under the direct influence of the MAW. It is evident that the sources of water and littoral pollution involve most of the entropic activities including the dumping of domestic wastes, sewage, agricultural wastes and industrial effluents into water bodies [23].

Various genera of fungi were isolated from metal-polluted environments [24], showing the ability to resist and to grow in the presence of toxic concentrations of heavy metals [25-26]. During this survey, the fungal strains isolated from the sandy contaminated environments exhibited a multi-tolerance behaviour. This selection is probably driven either by the most toxic elements or by more different metals acting synergistically [27]. The fungal biosorption of heavy metals varies from species to species but is also based on other factors [16]. On the other hand, despite the fact that it was isolated at **S3**, a distant site from the polluting outlets of the industrial area (Meboudja wadi and Seybouse wadi), *A.clavatus* showed a high tolerance to elevated concentrations of heavy metals. This suggests that some changes of the

dominant sea currents in the direction of the North-West side could be responsible for the dissemination of pollutants, including heavy metals, towards Ain Achir beach.

The results of this survey also revealed that most of the isolates were multi-resistant to Cu, Pb and Zn with the levels of resistance depending on the tested isolate. Species from the same genus did not have the same degree of tolerance as *A.terreus* showed more tolerance than *A.clavatus*. This difference in the metal tolerance may be due to the presence of one or more mechanisms of resistance and tolerance exhibited by each fungal strain [27]. Some studies [17,20] indicated that fungi species from various genera, and particularly of *Fusarium*, were isolated from contaminated soils, and were showing the ability to tolerate the presence of different heavy metals. Indeed, species of the genus *Aspergillus* and other tolerant fungal strains are also indicated having an elevated resistance to high levels of heavy metals concentrations [17, 28, 29, 30].

On the other hand, the toxic effect of each heavy metal increases with its rising concentration in the growth medium [31]. Despite the fact that Copper is an antifungal agent, the growth of *Aspergillus flavus* in the presence of high concentrations of Copper was reported in the literature [32]. The mechanism of tolerance to Copper is linked to its attachment to the surface absorption sites [33]. Zinc is a micronutrient and if adsorbed at high concentrations, the cells use a pathway to inhibit its uptake [34]. However, Lead has no metabolic relevance in fungi, and its uptake occurs through both intracellular and extracellular processes [4].

The term adaptation speed is an important armor that prompts one fungus more powerful than other fungi with higher MIC property. However, accumulation of excessive levels of these metals could be cytotoxic to the fungi [29].

Recent study, reporting on the strategies and uptake patterns for specific metal accumulations, indicated that these mechanisms may occur through the oxidation of organic acids tending with the uptake of some toxic metals [22,35]. Toxic metals such as Lead can destabilize the membrane structure and induce stress, generating the secretion of mucilaginous binding molecules that have a high affinity for metals [36] which physicochemical properties facilitate their uptake [37].

The highest uptake of Copper showed in this survey by *P.chrysogenum* indicated that having more binding sites on the cell wall, this fungus has also a biosorbent potential to remove Lead from the polluted environment. At 1600 mgl^{-1}, the uptake of heavy metal by the dead fungal strains can be explained by the uptake that occurs in the dead fungal cells because of the physic-chemical interactions between the metal ions and the negatively charged groups on the surfaces of dead cells [38]. On the other hand, it must be mentioned that during this work, most of the fungal strains exhibited changes in their morphology or in their medium with the formation of colourful mycelia due to the presence of heavy metals (mainly Zinc) as reported in the literature [39].

Finally, through the results of this investigation, it is evident that Mother Nature offers an alternative way to resolve some issues of the environmental pollution by heavy metals. The natural fungal strains, exhibiting a high tolerance against heavy metals such as Cooper, Lead and Zinc, can be used for the purification of contaminated environments. This latter point disserves a particular interest of further studies.

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Error Bounds for a Numerical Scheme with Reduced Slope Evaluations

Muhammad Yasir Ansari, Asif Ali Shaikh, Sania Qureshi

Department of Basic Sciences and Related Studies, Mehran University of Engineering and Technology,
Jamshoro, Sindh, Pakistan

ABSTRACT

In this paper, a new scheme of Runge-Kutta (RK) type has been discussed which utilizes only two slope evaluations per integration step while maintaining the third order accuracy as shown by the derived principal term of local truncation error. Characteristic stability polynomial is presented followed by the error analysis of the scheme. Error bounds in terms of Lotkin's bounds of the scheme have been derived and compared with the error bounds with existing standard schemes having similar number of slope evaluations as that of the scheme under consideration. Couples of numerical examples with varying nature are presented to test the performance of the developed scheme against some of the standard schemes having same number of slope evaluations per integration step.

KEYS WORDS: Initial value problems, Runge-Kutta scheme, autonomous and non-autonomous differential equations, Zero-stability.

1. INTRODUCTION

Ordinary Differential Equations (ODEs) have played a vital role in mathematical and biological sciences. Mathematical models based upon ordinary differential equations are very much important in various field of science including Biology (DNA molecules or biosynthesis phospholipids), Physics (Simple Pendulum), Chemistry (chemical reaction kinetics), Medicine (Pharmaceutical Drug Design), Population Dynamics (Verhulst-Pearl model), Engineering (beats of a vibrating system) and many more [1-5]. Mathematical modeling of ODEs is widely used in physical applications of above different kinds of indispensable areas. There are many problems of engineering and physical science which can be formulated into ordinary differential equations satisfying certain conditions (initial and/or boundary). If these conditions are prescribed for one and only point x_0 then such a problem together with the condition is known as an Initial Value Problem (IVP) as described in [6-8]. Main focus of the present paper is upon solving IVPs numerically with reduced slope evaluations required per integration step having discussion and derivation of the error bounds on the constant step size used for the numerical scheme under consideration.

Analytically, the solution to an initial value problem means finding an explicit expression for the unknown function $y(x)$. But analytical schemes are applicable only for selected class of IVPs mostly linear ones and a very few nonlinear IVPs. In cases when closed form solution does not exist, one has to go for the approximate solution using some numerical schemes as discussed in [9-10]. Various numerical schemes in past with different characteristics have been presented to solve such IVPs. In [11-15], authors have attempted to improve the order of accuracy of the existing standard linear RK type schemes whereas others in [16-18] have proposed new linear schemes with different characteristics. There is yet another group of scholars who have developed nonlinear schemes to solve those IVPs having rational solutions with some sort of singularity in them for which standard linear RK schemes do not perform well as can be consulted with [19-20]. Explicit and implicit or semi-implicit numerical schemes have also been derived to serve the purpose as shown in [21].

Consider an initial value problem in the form of

$$\frac{dy}{dx} = f\left(x, y(x)\right);\ y(x_0) = y_0,\ \ x \in \left[x_0, x_n\right] \tag{1}$$

where uniqueness of the solution of (1) has been assumed.

One of the most common numerical schemes for solving the equation (1) is the standard classical RK scheme as explained in [7]. Most efforts to increase the order of RK methods have been accomplished by increasing the number of terms of Taylor's series thus the number of function evaluations per integration step. Three number of function evaluations are required per integration step in the classical third order RK method. Many authors have attempted to increase the efficiency of RK method with lower number of function evaluations required as detailed in [22-26].

As a result, a third order RK type scheme has been proposed in [27] which requires only two function evaluations per integration step to solve only autonomous type of IVPs. In order to get a numerical scheme applicable for both autonomous and non-autonomous IVPs, an improved RK type numerical scheme was proposed in [25] which also employs two function evaluations per step while maintaining third order accuracy but the paper has not offered error analysis and bound on the step length (h) of the scheme. Taking inspiration from this research work, the present paper not only offers derivation of the scheme but also contains analysis of its local truncation error and corresponding error bounds.

Corresponding Author: Sania Qureshi, Department of Basic Sciences and Related Studies, Mehran University of Engineering and Technology, Jamshoro, Pakistan. Email: sania.qureshi@faculty.muet.edu.pk

The structure of the present paper has been organized as follows. In section 2, we presented the derivation of the third order improved RK scheme with two stages using the Taylor's series expansion. In section 3, the stability region of the scheme is discussed followed by the analysis of its Local Truncations Error (LTE) in section 4. Errors bounds of the scheme are further analyzed in section 5 whereas the results so obtained are discussed in the section 6 followed by section 7 for conclusion.

2. Derivation of the Improved RK Scheme

Consider the following structure of the proposed numerical scheme:

$$y_{n+1} = y_n + h(b_1 k_1 - b_{-1} k_{-1} + b_2(k_2 - k_{-2})) \tag{2}$$

$$k_1 = f\left(x_n, y_n\right)$$

$$k_{-1} = f\left(x_{n-1}, y_{n-1}\right)$$

$$k_2 = f\left(x_n + c_2 h, y_n + a_{21} k_1 h\right)$$

$$k_{-2} = f\left(x_{n-1} + c_2 h, y_{n-1} + a_{21} k_{-1} h\right)$$

with the assumptions that $c_2 = a_{21}$ because $c_i = \sum_{j}^{i-1} a_{ij}$ and $c_2 \in (0,1]$.

In the above equations, $c_2 = a_{21}, b_1, b_{-1}, b_2$ are the constants to be determined. Here k_1, k_{-1}, k_2, k_{-2} are the slopes to be Taylor expanded to get the proposed scheme in the following way:

$$k_1 = f$$

$$k_{-1} = f + \left(-f_x - f_y f\right)h$$
$$+ \frac{h^2}{2!}\left[f_{xx} + 2f_{xy}f + f_{yy}f + f_y^2 f + f_y f_x\right]$$
$$+ \frac{h^3}{3!}\begin{bmatrix} -f_{xxx} - 3f_{xxy}f - 3f_{xyy}f^2 - f_{yyy}f^3 - 4f^2 f_{yy}f_y - f_y^3 f \\ -f_x f_y^2 - 5f_{xy}f_y f - 3f_{yy}f_x f - f_{xx}f_y - 3f_{xy}f_x \end{bmatrix} + O\left(h^4\right)$$

$$k_2 = f + \left(f_x c_2 + f_y c_2 f\right)h + \left(\frac{1}{2}f_{y,y}f^2 c_2^2 + f_{x,y}fc_2^2 + \frac{1}{2}f_{x,x}c_2^2\right)h^2 +$$
$$\left(\frac{1}{6}f_{y,y,y}f^3 c_2^3 + \frac{1}{2}f_{x,y,y}f^2 c_2^3 + \frac{1}{2}f_{x,x,y}fc_2^3 + \frac{1}{6}f_{x,x,x}c_2^3\right)h^3 + O\left(h^4\right)$$

$$k_{-2} = f + \left(-f_x - f_y f + c_2 f_y f + c_2 f_x\right)h$$
$$+ \frac{h^2}{2!}\begin{bmatrix} f_{yy}f^2 c_2^2 + 2f_{xy}fc_2^2 - 2f_{yy}f^2 c_2 - 4f_{xy}fc_2 + f^2 f_{yy} + f_{xx}c_2^2 \\ -2f_y^2 fc_2 - 2f_x f_y c_2 + f_y^2 f + 2f_{xy}f - 2f_{xx}c_2 + f_x f_y + f_{xx} \end{bmatrix} +$$
$$+ \frac{h^3}{3!}\begin{bmatrix} -3f_{xxy}^3 f - 3f^2 f_{xyy} - 3f_{xy}f_x - f^3 f_{yyy} - f_y^3 f - f_y^2 f_x - f_{xx}f_y - 6f^2 f_{yy}f_y c_2 \\ -6f_{yy}f_x fc_2 - 6f_{xy}f_y fc_2 - 3f^3 f_{yyy}c_2^2 + 3f^3 f_{yyy}c_2 - 9f^2 f_{xyy}c_2^2 + f_{yy}f_x fc_2 \\ +15f_{xy}f_y fc_2 + 9f^2 f_{xyy}c_2 - 15f_{xy}f_y f - 4f^2 f_{yy}f_y - 3f_{yy}f_x f - f_{xxx} \\ +3f^2 f_y f_{yy}c_2 + 3f_y^3 fc_2 + 3f_y^2 f_x c_2 + 3f_{xx}f_y c_2 - 6f_{xy}f_x c_2^2 - 9ff_{xxy}^2 c_2^2 + 9f_{xy}f_x c_2 \\ +9f_{xxy}fc_2 + 3f^2 f_{xyy}c_2^3 + 3ff_{xxy}c_2^3 + f^3 f_{yyy}c_2^3 - 3f_{xxx}c_2^2 + 3f_{xxx}c_2 + f_{xxx}c_2^3 \end{bmatrix} + O\left(h^4\right)$$

$$y_{n+1} = y_n + hf(b_1 - b_{-1}) + h^2(f_x + f_y f)(b_{-1} + b_2) + \frac{1}{2}h^3 \begin{bmatrix} f_{xx}(-b_{-1} + 2b_2c_2 - b_2) + 2f_{xy}f(-2b_{-1} + 4b_2c_2 - 2b_2) \\ + f_{yy}f^2(-b_{-1} + 2b_2c_2 - b_2) + f_y^2 f(-b_{-1} + 2b_2c_2 - b_2) \\ + f_y f_x(-b_{-1} + 2b_2c_2 - b_2) \end{bmatrix}$$

$$\frac{h^4}{6} \begin{bmatrix} f_{xxy}f(3b_2 + 9b_2c_2^2 - 9b_2c_2 + 3b_{-1}) + f^2 f_{xyy}(3b_2 + 9b_2c_2^2 - 9b_2c_2 + 3b_{-1}) + f_{xy}f_x(3b_2 + 6b_2c_2^2 - 9b_2c_2 + 3b_{-1}) \\ + f_{yyy}^3 f(b_2 + 3b_2c_2^2 - 3b_2c_2 + b_{-1}) + f f_y^3(b_2 - 3b_2c_2 + b_{-1}) + f_y^2 f_x(b_2 - 3b_2c_2 + b_{-1}) + f_{xx}f_y(b_2 - 3b_2c_2 + b_{-1}) \\ f_{yy}f_y f^2(6b_2c_2^2 + 4b_2 - 12b_2c_2 + 4b_{-1}) + f_{yy}f_x f(6b_2c_2^2 + 9b_2c_2 + 3b_2 + 3b_{-1}) \\ + f_{xy}f_y f(6b_2c_2^2 - 15b_2c_2 + 5b_2 + 5b_{-1}) + f_{xxx}(3b_2c_2^2 - 3b_2c_2 + b_2 + b_{-1}) \end{bmatrix} \quad (3)$$

Generally, the Taylor's series for a function $y(x_n + h)$ is as follows:

$$y(x_n + h) = y(x_n) + hf + \left(\frac{1}{2}f_x + \frac{1}{2}f_y f\right)h^2 +$$

$$\left(\frac{1}{6}f_{x,x} + \frac{1}{3}f_{x,y}f + \frac{1}{6}f^2 f_{y,y} + \frac{1}{6}f_y^2 f + \frac{1}{6}f_x f_y\right)h^3 + \quad (4)$$

$$\left(\begin{matrix} \frac{1}{24}f_{x,x,x} + \frac{1}{8}f_{x,x,y}f + \frac{1}{8}f_{x,y,y}f^2 + \frac{5}{24}f_y ff_{x,y} + \frac{1}{8}f_x f_{x,y} + \frac{1}{24}f_{y,y,y}f^3 + \\ \frac{1}{6}f_y f^2 f_{y,y} + \frac{1}{8}f_x ff_{y,y} + \frac{1}{24}f_y^3 f + \frac{1}{24}f_x f_y^2 + \frac{1}{24}f_y f_{x,x} \end{matrix}\right)h^4 + O(h^5)$$

Comparing the equations (3) and (4) up to h^3 terms, the following order conditions are obtained:

$$b_1 - b_{-1} = 1, \ b_{-1} + b_2 = \frac{1}{2}, \ -b_{-1} + 2b_2c_2 - b_2 = \frac{1}{6} \quad (5)$$

After solving this system of nonlinear algebraic equations, we have the following general structure where c_2 be a free parameter:

$$b_{-1} = \frac{1}{12}\frac{-5 + 6c_2}{c_2}, \ b_1 = \frac{1}{12}\frac{-5 + 18c_2}{c_2}, \ b_2 = \frac{5}{12c_2} \quad (6)$$

After trying various values of the free parameter c_2, we have come up with following numerical scheme which employs two slope evaluations per integration step while maintaining the third order accuracy as shown by the local truncation error in the next section:

$$\left.\begin{matrix} y_{n+1} = y_n + \frac{h}{4}\left[k_1 + 3k_{-1} + 5(k_2 - k_{-2})\right] \\ k_1 = f(x_n, y_n); \ k_{-1} = f(x_{n-1}, y_{n-1}) \\ k_2 = f\left(x_n + \frac{1}{3}h, y_n + \frac{1}{3}k_1 h\right); \ k_{-2} = f\left(x_{n-1} + \frac{1}{3}h, y_{n-1} + \frac{1}{3}k_{-1}h\right) \end{matrix}\right\} \quad (7)$$

3. Error Analysis

For getting the expression of local truncation error of the proposed numerical scheme, a functional related to the scheme is considered:

$$L(z(x), x) = z(x_{n+1}) - \text{Improved RK Scheme shown by } (7)$$

where $z(x)$ is a function arbitrary in nature and can be differentiated as many times as required on $[x_0, x_n]$. Having Taylor expanded the above equation about x, it is observed that the all terms up to h^3 have been cancelled and thus the local truncation error comes out to be as follows:

$$LTE = \frac{h^4}{72}\begin{bmatrix} 7\left(f_{xxx} + f_{yyy}f^3\right) + 21\left(f_{xxy}f + f_{xyy}f^2\right) + \\ 12\left(f_y^3 f + f_x f_y^2 + f_{xx}f_y\right) + \\ 26\left(f_{yy}f_x f + f_{xy}f_x\right) + 50 f_{xy}f_y f + 38 f^2 f_{yy}f_y \end{bmatrix} + O\left(h^5\right) \tag{8}$$

Further, being at least third order accurate the developed numerical scheme (7) is also **consistent**.

4. Stability Analysis

The stability of the developed numerical scheme in (7) is checked using it to the Dahlquist's test problem [28] as follows:

$$y'(x) = \lambda y(x); \ \mathrm{Re}(\lambda) < 0 \tag{9}$$

The slopes involved in the scheme become

$$k_1 = \lambda y_n, \ k_{-1} = \lambda y_{n-1}$$

$$k_2 = \lambda\left(1 + \frac{h\lambda}{3}\right)y_n, \ k_{-2} = \lambda\left(1 + \frac{h\lambda}{3}\right)y_{n-1}$$

After simplification, we get the following stability polynomial

$$\rho(\omega, z) = \omega^2 + p(z)\omega + q(z) \tag{10}$$

where $p(z) = -\frac{1}{12}\left(5z^2 + 18z + 12\right)$, $q(z) = \frac{1}{12}\left(5z^2 + 6z\right)$, and $z = h\lambda$. Further, ω_1 and ω_2 are said to be the zeros of the stability polynomial (10).

For analyzing stability and obtaining stability region of the developed numerical scheme, we employ **Schur – Cohn stability criterion** discussed in [29]. For this purpose, we define:

$$\overline{\rho}(\omega, z) = q'\omega^2 + p'\omega + 1 \ \text{ and } \ \overline{\rho}_1(\omega, z) = \frac{1}{\omega}\left[\overline{\rho}(0, z)\rho(\omega, z) - \rho(0, z)\overline{\rho}(\omega, z)\right]$$

where p' and q' are complex conjugates of p and q respectively. Here, $\overline{\rho}_1(\omega, z)$ is at least of first degree polynomial as shown below:

$$\overline{\rho}_1(\omega, z) = \frac{1}{\omega}\left[\omega^2 + p\omega + q - q\left(q'\omega^2 + p'\omega + 1\right)\right] = \omega + p - qq'\omega - qp'$$

By Schur-Cohn theorem, $|\omega_1| < 1$ and $|\omega_2| < 1$ if and only if $|q| < 1$ and $|p - qp'| + |q|^2 < 1$.

Region of the stability for the developed numerical scheme is sketched (unshaded region) below along-with its interval of stability:
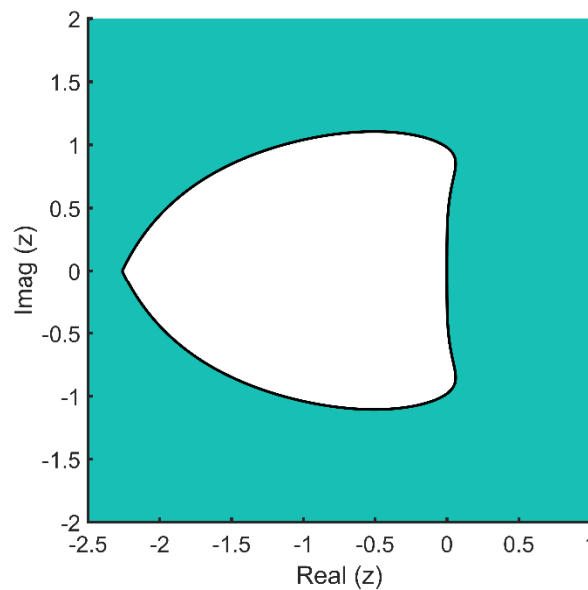

Figure 1. Region of stability with interval of stability being $\left(-2.248, 0.055\right)$ in the Complex Plane

This completes the proof of stability of the developed numerical scheme. Moreover, the first characteristic polynomial $\rho(z) = 0$, has two zeros, that is, $\omega = 0, 1$; it can be claimed that the scheme is **zero – stable**. Further, depending upon the above discussion of consistency and stability the convergence of the numerical scheme is analyzed in the following way:

Theorem (Dahlquist's Equivalence Theorem)
As reported in [30]; "For a linear multistep numerical scheme consistent with an ordinary differential equation $y'(x) = f(x, y(x))$; where $f(x, y(x))$ is assumed to satisfy the Lipchitz condition, zero-stability and consistency are necessary and sufficient conditions for convergence."
Mathematically,

$$\text{Consistency+Zero-Stability} \iff \text{Convergence}$$

This theorem guarantees for the developed scheme to be **convergent**.
As far as order of accuracy of the scheme is concerned; it has been revealed while deriving the scheme that it takes three order conditions (5) and utilizes Taylor's expansion up to the term containing h^3. This clearly shows that global error involved in the method is of order **three**, that is, global truncation error $= O(h^3)$ hence the order of the scheme.

5. Propagation of Errors

It is a common fact that numerical solution of an ordinary differential equation contains round off (uncontrollable) and truncation (discretization) errors where truncation error is generally of two types, that is; local and global truncation errors, which remain under control of the analyst.

Detailed study of magnitude and characteristics of truncation error is important to accept any new devised iterative method. In order to be of some use, an iterative must discuss error bounds it contains as claimed in [31]. The developed numerical scheme (7) can easily be compared with Taylor series expansion of the form:

$$y(x_n + h) = y_n + hf + \frac{h^2}{2}(f_x + f_y f) + \frac{h^3}{6}(f_{xx} + 2f_{xy}f + f_{yy}f^2 + (f_y)^2 f + f_y f_x)$$

$$+ \frac{h^4}{24}\left(\begin{array}{c} f_{xxx} + 3f_{xxy}f + 3f_{xyy}f^2 + f_{yyy}f^3 + 4f^2 f_{yy}f_y \\ + f_y^3 f + f_x f_y^2 + 5f_{xy}f_y f + 3f_{yy}f_x f + f_{xx}f_y + 3f_{xy}f \end{array}\right) + R_n \tag{11}$$

where $R_n = \frac{y^{(n+1)}(\eta)}{(n+1)!}h^{n+1}$, $x_k < \eta < x_{k+1}$

The terms containing h^4 have been truncated while developing the modified iterative method resulting the bound of local truncation error given by:

$$LTE \leq \max_{k=\{01,2,\cdots,N\}} \frac{h^4}{4!} y^{(iv)}(\eta) \text{ for } x_k < \eta < x_{k+1}$$

According to above inequality, we find that local truncation error is proportional to the power 4 of the step size and fourth derivative of the given differential equation. Likewise, global truncation error will be $GTE = O(h^3)$. It implies that halving the step size will decrease the error by a factor of about $1/8$. In order to prove it in general sense; error bound for local truncation error (LTE) is computed using Lotkin's Error Bounds discussed by Lotkin in [32]. From (8), the bound for the local truncation error is obtained as:

$$\left|\psi(x_k, y_n)h^4\right| < \frac{29}{9}h^4 P^3 Q \tag{12}$$

where $\psi(x_k, y_n)$ is known as *Principal Error Function* for the proposed method and *P, Q* are positive constants given by Lotkin as:

$$\left|f(x, y)\right| < Q \text{ and } \left|\frac{\partial^{i+j} f}{\partial x^i \partial y^j}\right| < \frac{P^{i+j}}{Q^{j-1}}; \; (i+j) \leq \text{order of the scheme}$$

Following table shows error bounds, number of function evaluations and order of accuracy of some numerical schemes compared with that of the developed scheme (7). The Table 1 reveals strength of the developed scheme in connection with order of accuracy in particular. The table also shows that the third order standard linear Ralston's scheme will take comparatively larger step size with three function evaluations per integration step whereas the scheme developed exploits only two.

Table 1. Error Bounds

Method	Error Bound on LTE	Function Evaluations	Order of Accuracy
Improved Euler	$\left\|\psi\left(t_n,y_n\right)h^3\right\| < \dfrac{2}{3}h^3 P^2 Q$	TWO	2
Midpoint Euler	$\left\|\psi\left(t_n,y_n\right)h^3\right\| < \dfrac{1}{2}h^3 P^2 Q$	TWO	2
Ralston	$\left\|\psi\left(t_n,y_n\right)h^3\right\| < \dfrac{5}{12}h^3 P^2 Q$	TWO	2
Ralston	$\left\|\psi\left(t_n,y_n\right)h^4\right\| < 0.1111 h^4 P^3 Q$	THREE	3
Proposed	$\left\|\psi\left(t_n,y_n\right)h^4\right\| < \dfrac{29}{9}h^4 P^3 Q$	**TWO**	3

6. RESULTS AND DISCUSSION

For testing the developed numerical scheme, couples of initial value problems of varying nature are considered from the literature. Standard linear numerical schemes having same number of slope evaluations per integration step are selected for comparison with the developed scheme. To serve the purpose, maximum absolute error along the integration interval $\left(E = \max\limits_{n=1,2,\cdots,N} \left| y\left(x_n\right) - y_n \right| \right)$, final absolute global error $\left(E\left(t = x_n\right) = \left| y\left(x = N\right) - y_N \right| \right)$ and CPU time values have been tabulated for all the schemes under consideration. Each data cell in every table of the numerical experiments lists the maximum absolute error, final absolute global error and the CPU time values from top to bottom order. In all types of initial value problems under consideration in the present paper, the developed scheme proposed here yields smaller errors in comparison with other schemes having same order of local accuracy. The graphs for this purpose tell the similar sort of story even though considerably small step size is chosen to solve the underlying IVP as depicted in the numerical experiments discussed below.

Example 1. In this first problem, a linear initial value problem is chosen which is given as:

$$\frac{dy}{dx} = x + y, \ y\left(0\right) = 1$$

whereas its exact analytical solution is given by:

$$y\left(x\right) = -x - 1 + 2e^x$$

It is observed from the Table 2 that with the increasing number of integration steps, maximum absolute error on the integration interval $\left[0,1\right]$ and the final absolute global error are decreasing for each numerical scheme under consideration with the developed scheme (7) having the smallest errors among all. CPU time values exhibit similar type of trend for almost every scheme in the table.

Table 2. Errors and CPU values for Example 1

Scheme/NI	64	128	256	512	1024
Proposed	3.3760e-06	4.2703e-07	5.3693e-08	6.7313e-09	8.4264e-10
	3.3760e-06	4.2703e-07	5.3693e-08	6.7313e-09	8.4264e-10
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00
Heun's	2.1863e-04	5.4980e-05	1.3785e-05	3.4514e-06	8.6349e-07
	2.1863e-04	5.4980e-05	1.3785e-05	3.4514e-06	8.6349e-07
	0.0000e+00	0.0000e+00	0.0000e+00	1.5625e-02	1.5625e-02
Ralston	2.1863e-04	5.4980e-05	1.3785e-05	3.4514e-06	8.6349e-07
	2.1863e-04	5.4980e-05	1.3785e-05	3.4514e-06	8.6349e-07
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00

Example 2. Once again, a linear initial value problem is considered but it has the slope with a transcendental function given on the right:

$$\frac{dy}{dx} = 6\sin\left(2x\right) - 20y\left(x\right), \ y\left(0\right) = 1$$

Its exact analytical solution is given by:

$$y\left(x\right) = -\frac{3}{101}\cos\left(2x\right) + \frac{30}{101}\sin\left(2x\right) + \frac{104}{101}e^{-20x}$$

It can be seen from the Table 3 that with the increasing number of integration steps, maximum absolute error on the integration interval $\left[0,1\right]$ and the final absolute global error are decreasing for each numerical scheme under consideration with the

developed scheme (7) having the smallest errors among all. CPU time values exhibit similar type of trend for almost every scheme in the table.

Table 3. Errors and CPU values for Example 2

Scheme/NI	64	128	256	512	1024
Proposed	1.9368e-03	2.4081e-04	3.0074e-05	3.7624e-06	4.7041e-07
	4.2495e-08	6.1629e-09	8.3113e-10	1.0792e-10	1.3750e-11
	6.2500e-02	0.0000e+00	0.0000e+00	0.0000e+00	1.0469e+00
Heun's	7.8433e-03	1.7335e-03	4.0882e-04	9.9209e-05	2.4442e-05
	8.3179e-05	1.9013e-05	4.5582e-06	1.1166e-06	2.7639e-07
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00
Ralston	7.8442e-03	1.7338e-03	4.0888e-04	9.9224e-05	2.4445e-05
	5.5931e-05	1.2780e-05	3.0633e-06	7.5036e-07	1.8571e-07
	1.5625e-02	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00

Example 3. In this example, a linear initial value problem is under discussion whose exact solution is not available in terms of elementary mathematical functions:

$$\frac{dy}{dx} = 2xy(x) - 1, \ y(0) = 1$$

The exact solution consists of a special function called the Gauss error function [Temme, N. M, 2010]

$$y(x) = \left(-\frac{1}{2}\sqrt{\pi}\ erf(x) + 1\right)e^{x^2}$$

It can be seen from the Table 4 that with the increasing number of integration steps, maximum absolute error on the integration interval $[0,1]$ and the final absolute global error are decreasing for each numerical scheme under consideration with the developed scheme (7) having the smallest errors among all. CPU time values exhibit similar type of trend for almost every scheme in the table.

Table 4. Errors and CPU values for Example 3

Scheme/NI	64	128	256	512	1024
Proposed	8.2727e-06	1.0554e-06	1.3326e-07	1.6741e-08	2.0978e-09
	8.2727e-06	1.0554e-06	1.3326e-07	1.6741e-08	2.0978e-09
	0.0000e+00	0.0000e+00	3.7500e-01	6.7188e-01	1.1406e+00
Heun's	1.6085e-04	4.0053e-05	9.9932e-06	2.4958e-06	6.2363e-07
	1.6085e-04	4.0053e-05	9.9932e-06	2.4958e-06	6.2363e-07
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00
Ralston	9.2021e-05	2.3037e-05	5.7634e-06	1.4414e-06	3.6040e-07
	9.2021e-05	2.3037e-05	5.7634e-06	1.4414e-06	3.6040e-07
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00

Example 4. Here, a nonlinear autonomous initial value problem is under consideration:

$$\frac{dy}{dx} = y(x)(1 - y(x)), \ y(0) = 0.5$$

The exact solution of which is provided by: $y(x) = \dfrac{1}{1 + e^{-x}}$

It can be seen from the Table 5 that with the increasing number of integration steps, maximum absolute error on the integration interval $[0,1]$ and the final absolute global error are decreasing for each numerical scheme under consideration with the developed scheme (7) having the smallest errors among all. CPU time values exhibit similar type of trend for almost every scheme in the table. Further, the developed numerical scheme is also tested with considerably fewer number of integration steps (NI=20) against other methods but still found to be better in terms of absolute errors as shown by the Figure 2.

Table 5. Errors and CPU values for Example 4

| Scheme/NI	64	128	256	512	1024
Proposed	3.8438e-08	4.8357e-09	6.0639e-10	7.5920e-11	9.4965e-12
	3.8438e-08	4.8357e-09	6.0639e-10	7.5920e-11	9.4965e-12
	7.8125e-02	1.2500e-01	4.8438e-01	5.1563e-01	1.0000e+00
Heun's	2.4671e-06	6.1522e-07	1.5361e-07	3.8378e-08	9.5915e-09
	2.4671e-06	6.1522e-07	1.5361e-07	3.8378e-08	9.5915e-09
	1.5625e-02	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00
Ralston	6.0860e-07	1.5184e-07	3.7923e-08	9.4758e-09	2.3683e-09
	6.0860e-07	1.5184e-07	3.7923e-08	9.4758e-09	2.3683e-09
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00
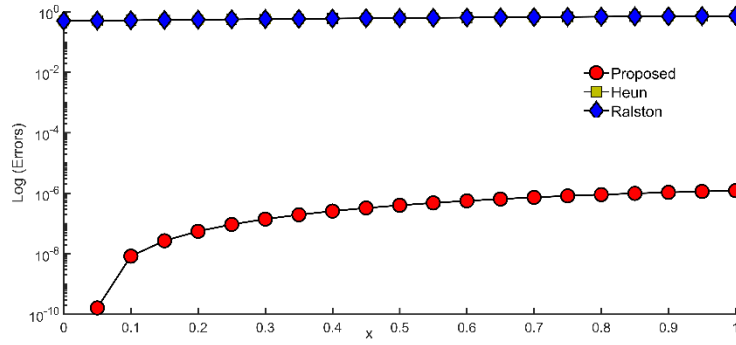
Figure 2. Absolute Errors with NI=20 for Example 4

Example 5. Finally, a nonlinear initial value problem with no solution in closed-form has been selected as follows:

$$\frac{d}{dx} y(x) = x^2 - y(x)^2, \ y(0) = 0$$

whose solution has been obtained using Maple symbolic environment as shown below:

$$y(x) = \begin{cases} 0 & x = 0 \\ \dfrac{x\left(\mathrm{BesselI}\left(-\dfrac{3}{4},\dfrac{1}{2}x^2\right)\sqrt{2}\pi - 2\mathrm{BesselK}\left(\dfrac{3}{4},\dfrac{1}{2}x^2\right)\right)}{\sqrt{2}\mathrm{BesselI}\left(\dfrac{1}{4},\dfrac{1}{2}x^2\right) + 2\mathrm{BesselK}\left(\dfrac{1}{4},\dfrac{1}{2}x^2\right)} & \text{otherwise} \end{cases}$$

where $\mathrm{BesselI}(v,z)$ and $\mathrm{BesselK}(v,z)$ are the Bessel functions of the first and second kind respectively.

It can be seen from the Table 6 that with the increasing number of integration steps, maximum absolute error on the integration interval $[0,1]$ and the final absolute global error are decreasing for each numerical scheme under consideration with the developed scheme (7) having the smallest errors among all. CPU time values exhibit similar type of trend for almost every scheme in the table. Further, the developed numerical scheme is also tested with considerably fewer number of integration steps (NI=20) against other methods but still found to be better in terms of absolute errors as shown by the Figure 3.

Table 6. Errors and CPU values for Example 5

| Scheme/NI	64	128	256	512	1024
Proposed	1.0483e-06	1.3285e-07	1.6720e-08	2.0972e-09	2.6259e-10
	1.0483e-06	1.3285e-07	1.6720e-08	2.0972e-09	2.6259e-10
	0.0000e+00	0.0000e+00	5.6250e-01	6.5625e-01	9.3750e-01
Heun's	3.7620e-05	9.3566e-06	2.3331e-06	5.8251e-07	1.4553e-07
	3.7620e-05	9.3566e-06	2.3331e-06	5.8251e-07	1.4553e-07
	0.0000e+00	0.0000e+00	4.6875e-02	0.0000e+00	0.0000e+00
Ralston	8.3089e-06	2.0616e-06	5.1343e-07	1.2811e-07	3.1996e-08
	8.3089e-06	2.0616e-06	5.1343e-07	1.2811e-07	3.1996e-08
	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00	0.0000e+00
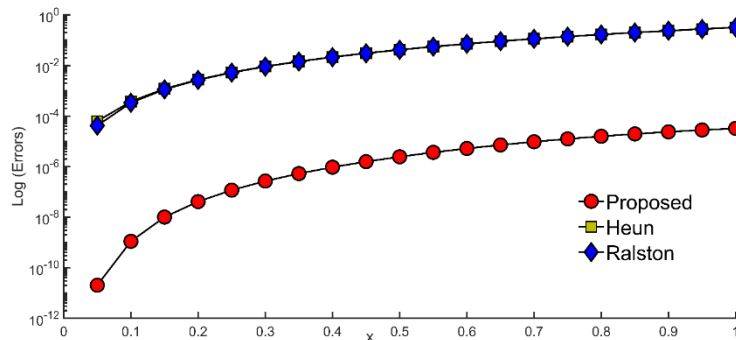
Figure 3. Absolute Errors with NI=20 for Example 5

7. CONCLUSION

The present work demonstrates the efficiency of an improved version of RK type scheme having third order accuracy especially in terms of number of slope evaluations per integration step and the error bound on the step length of the scheme. Errors produced by the presented scheme are much smaller than the errors given by other schemes taken for consideration. Although, the step length is taken to be as large as 0.05 but the curve of absolute errors of the scheme remains below the error curves of the other schemes considered for comparison. In addition to this, the presented scheme has error bound for which one may easily check the number of iterations required by the scheme before to actually employing it on an initial value problem. This has been shown that the scheme requires fewer number of integration steps in comparison with other schemes as given in the first table. In the future, the present research work is planned to be stretched for the derivation of a fourth order RK type scheme with three number of slope evaluations per integration step keeping its errors in control with the idea of the error bounds on its step length.

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Performance Evaluation on Chemical and Biological Methods, Used to Reduce the Damage Caused by Chickpea Pod Borer (*Helicoverpa armigera*)

H. Miri*, G.R. Mohammadi, M.E. G. Hobadi, N. Moini Naghade

Department of Crop Production and Plant Breeding, College of Agriculture and Natural Resources,
Razi University, Kermanshah, Iran

ABSTRACT

In order to compare the efficiency of chemical and biological methods, used to reduce the damage caused by chickpea pod borer, an experiment was conducted as a randomized complete block design with three replications, at the college of Agriculture and Natural Resources, Razi University, Kermanshah, Iran, during March to June, 2013. Treatments consisted of Bacillus thuringiensis (Bt) insecticide, release of Bracon hebetor wasps at large larval stage of the pest, release of the wasps at medium larval stage of the pest, spraying Sevin (chemical control), and control (no control method). Results showed that, control treatment had the highest harvest index and seed protein content. Also, the highest amounts of traits including seed yield, number of pods per plant, number of seeds per pod, biological yield, plant height, leaf chlorophyll and hundred-seeds weight, were obtained for the treatments chemical control, and release of Bracon wasps at large and medium larval stages of chickpea pod borer, respectively. Chemical control treatment had the highest inhibitory effect, on controlling the chickpea pod borer pest population, although the use of Bracon wasps can be considered as an effective and ecological friendly method to reduce pod borer infestation in chickpea.

KEYWORDS: Bracon wasp; Pod borer; Biological control

INTRODUCTION

Chickpea (Cicer arietinum L.) is an important grain legume crop, belonging to the family Fabaceae. Because of high nutritive value, chickpea is well considered, and is modified for some traits including protein, carbohydrate, and cholesterol lowering fiber, oil, ash, calcium and phosphorus. Chickpea cultivation raises the growers' income and contributes to soil fertility(Younis, Iqbal, Farooq, Jamil, & Khan, 2015). The crop grows generally under moderate to cold and semi-arid climates of the country, with a highly variable precipitation(Soltani, Ghassemi-Golezani, Khooie, & Moghaddam, 1999). The pod borer, Helicoverpa armigera Heubn is the most economically important pest of chickpea(Abbasi et al., 2007), which causes a significant damage to many farms, vegetables and crops. It feeds generally on flower buds, flowers and bolls. Females place eggs on the flowering and fruiting structures of these crops, where hungry larval feeding leads to huge economic loss (Cunningham et al., 1999).

Biological control is the use of living organisms to keep pest populations at lower damaging levels. Natural enemies of arthropods fall into three main classes: predators, parasitoids, and pathogens. Biological control is often more effective, when it is joined with other pest control strategies, in an integrated pest management (IPM) program. Bacillus thuringiensis (BT) is a Gram-positive, soil-dwelling bacterium, usually used as a biological insecticide. BT is mostly used in agriculture, especially in organic farming. Bt is safe for humans and is used in urban aerial spraying programs, and in transgenic crops(Ibrahim, Griko, Junker, & Bulla, 2010). The microbial (Bt based) insecticides can be used as component of integrated pest management(IPM) approach to provide an environmentally safe and suitable alternative to generally hazardous, broad spectrum chemical insecticides used against H. armigera. As far as environmental protection is concerned, there is need for complimentary use of microbial (Bt based) and botanical insecticides in support of IPM. Biologically derived insecticides, such as Bt-basedbio-pesticide have provided a commercial alternative to broad-spectrum chemical insecticide because of their specificity in killing target pest(Khalique Ahmed, Khalique, Durrani, & Pitafi, 2012). Bracon hebetor is a public gregarious ecto-larval parasitoid, which belongs to the family Braconidae (Super family Ichneumonoidea). There appears to be two strains of parasitoid, one attacking field pests, and the other predates the pests of stored products. B. hebetor larvae grow by rasping a cavity through the host's integument and feeding on pest tissues. Parasitism occurs during the year Aim of

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*Corresponding author: H. Miri, Department of Crop Production and Plant Breeding, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran. E-mail: hamze_miri@yahoo.com

present experiment is to compare the performance of chemical and biological methods, used to reduce damage of the chickpea pod borer (Helicoverpa armigera), in Kermanshah west Iran.

MATERIALS AND METHODS

The experiment was conducted as a randomized complete block design with three replications, at Agriculture and Neutral Resources College of Razi University, Kermanshah (Latitude: 34°18′51″N, 47°03′54″E; altitude: 4557 ft.), Iran, during March to June, 2013. Treatments consisted of BT insecticide, release of the wasps at large larval stage, release of the wasps at medium larval stage, spraying (chemical control), and control plot. The treatments chemical control by Sevin (250ml acre-1), and BT insecticide (1 kg/ha), were applied simultaneously (before flowering), medium larvae were released at the 50% flowering, and release of large larvae was performed at the beginning of pod formation. In order to measure the traits, fortnightly sampling was begun on early May. Leaf SPAD value was measured on three randomly selected plants in each plot (Japan, Minolta, and SPAD-502). Plant height was determined on three randomly selected plants in each plot. Final harvesting was conducted on two middle rows at each plot (1m2). Harvested plants were weighted after drying, to measure the biological yield. In order to obtain the seed yield, five plants were randomly harvested from each plot, and the number of pods and seeds per plant were counted. To determine the weight of 100 seeds, four samples of 100 seeds were counted, weighted, and their means were calculated. Seeds belong to the harvested plants from each plot were separated and weighted. Measuring seed protein content was performed using volumetric balloon.

Staging the fight against chickpea pod borer

Plant growth and pest conditions from germination until completely pod maturity stage, were monitored on a regular weekly basis, and data were collected. To determine the Bracon hebator flight peak on April 25, a pheromone trap was installed in the field, and data from trapped wasps were gathered once every two days. Then, according to the flight peak, while the maximum number of small larvae were observed, farm was sprayed at a rate of 2 to 3 kg per hectare, using BT microbial toxins (Strain in use: Bacillus thuringiensis serotype H-3a3b), and after 12 days spraying, once every two days, data were collected from the experimental plots. Also, at the medium and large larval stages, 2000 Bracon wasps consisting of 80% female and 20% male, were released on the predetermined experimental plots, and data were collected for 12 days once every two days on the experimental plots after releasing the wasps. In order to avoid the flight of wasps on other treatment plots, the plots were bordered using thin meshes, with no inhibition for normal functioning of the wasps. Samples were also collected from chemical control (with Sevin pesticide at a rate of 3 kg per hectare) and control plots. To determine the number of infected and non-infected capsules, three plants of each plot were selected randomly, and marked, before applying the treatments, and then the number of pods was counted. To study the parasite damage, 12 days after the application of the treatments, the number of infected and non-infected capsules was counted again. Data analyses were carried out using SAS software (SAS Institute 2003). The Means were separated using LSD test at the probability level of 5 percent.

RESULTS

Analysis of variance indicated that, the treatments had significant effects on biological yield, 100-seeds weight, plant height, and seed protein content at 5% probability level, seed yield, leaf chlorophyll, number of pods per plant, number of seeds per pod, and harvest index at 1% probability level (Table 1).

Table 1. Variance Analysis of the Impact of Pod Borer Larvae on Chickpea Traits

SOURCES OF VARIANCE	DF	BIOMASS	NUMBER OF PODS PER PLANT	NUMBER OF SEEDS PER POD	SEED WEIGHT	LEAF CHLOROPHYLL	PLANT HEIGHT	PROTEIN CONTENT	SEED YIELD	HI
BLOCK	2	1998.296[NS]	17.532[*]	0.030[NS]	20.220[NS]	38.438[NS]	7.108[NS]	13.471[NS]	66.445[NS]	0.002[NS]
TREATMENT	4	9005.729[*]	55.699[**]	0.408[**]	103.335[*]	129.280[**]	83.852[*]	24.410[*]	1220.892[**]	0.013[**]
ERROR	8	16263.034	24.621	0.157	232.078	124.797	122.856	42.348	449.285	0.009
CV (%)		16.571	11.382	9.071	15.930	10.527	15.243	10.906	6.139	7.214

Table 2. Effect of Applying Different Treatments on Number and Situation of the Pods per Plant

TREATMENT	TREATMENT APPLICATION			
	BEFORE TREATING		AFTER TREATING	
	HEALTHY PODS	INFECTED PODS	HEALTHY PODS	INFECTED PODS
CHEMICAL CONTROL	14	6	24	2
RELEASE OF BRACON WASP PARASITOID AT LARGE LARVAL STAGE	13	6	21	4
RELEASE OF BRACON WASP PARASITOID AT MEDIUM LARVAL STAGE	13	7	19	6
BT INSECTICIDES	11	6	17	8
CONTROL	12	7	14	11

Biological yield and Number of pods per plant

The highest and the lowest chickpea biological yield were related to the chemical control, and control treatments, respectively. No significant difference was found between the treatments release of bracon wasps at large and medium larval stages with chemical control. Results also indicated that, the treatments release of bracon wasp showed a higher biological yield compared with BT treatment (Figure 1).
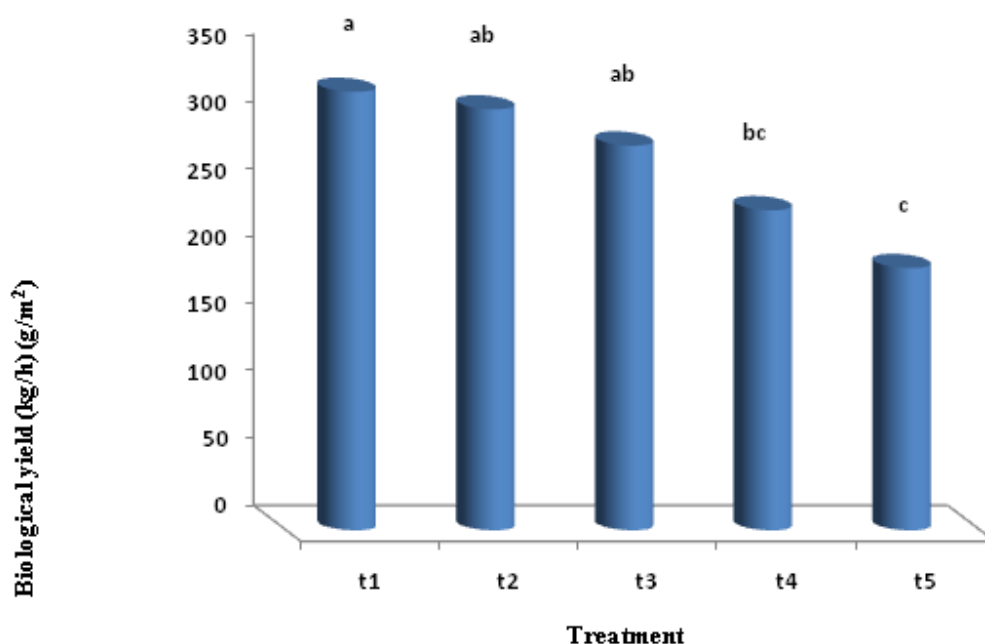
Figure 1. Biological yield of chickpea at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

According to the results, the highest number of pods per plant was observed at the chemical control treatment. Also, there was no significant difference between the treatments chemical control and release of bracon wasps on large larvae, in terms of this trait. Release of wasp's at large larval stage had a higher number of pods per plant, compared to BT treatment. The difference between the treatments of BT insecticide and releasing the wasps at medium larval stage was not significant for this trait (Figure 2).
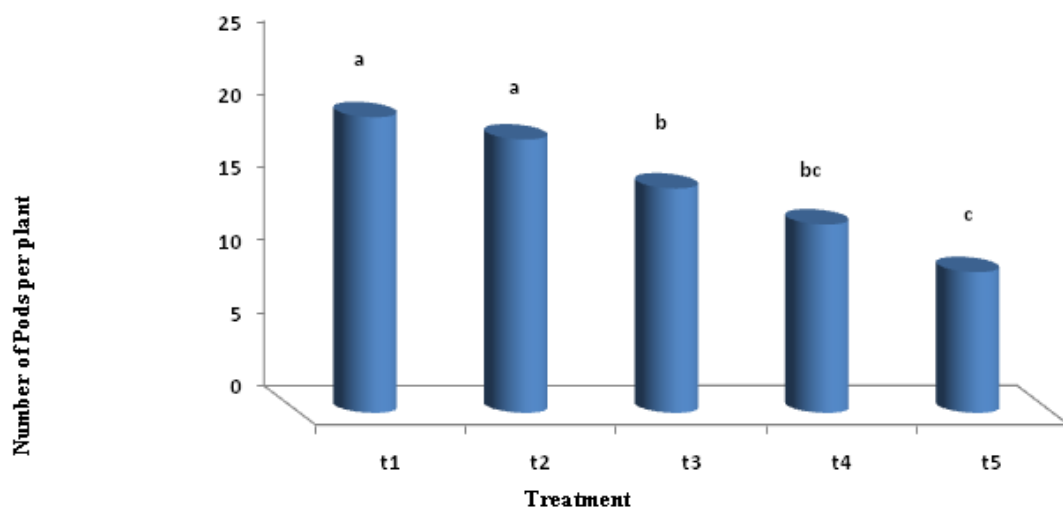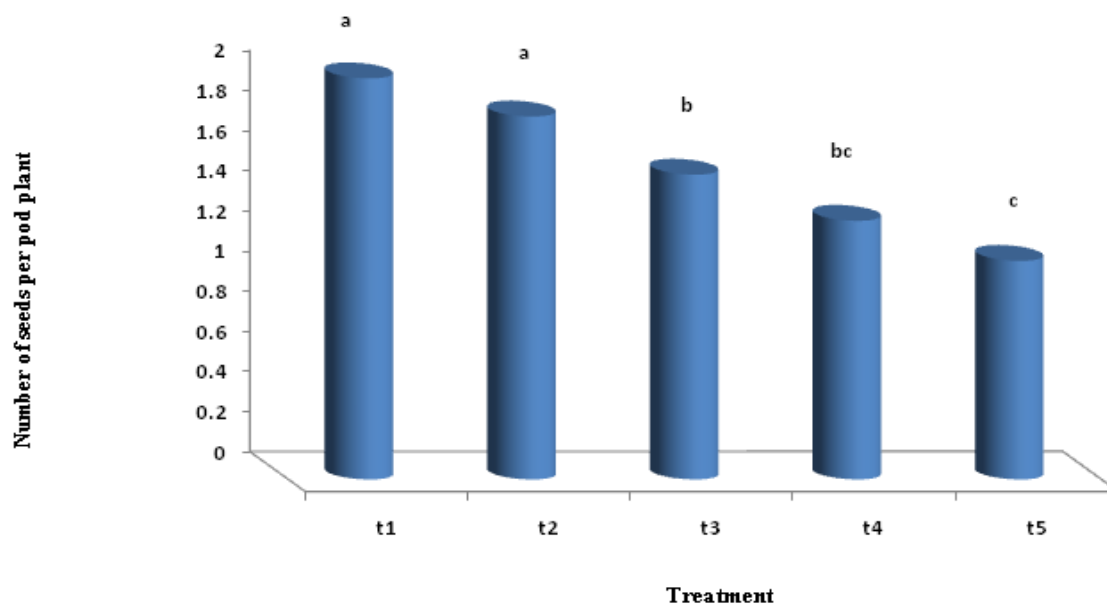
Figure 2. Number of pods per chickpea's plant at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

Number of seeds per plant, and Hundred-seed weight
Based on the results, the minimum and the maximum number of seeds per pod were related to the treatments of control and chemical control treatments, respectively. Considering the number of seeds per pod, there was no significant difference between control and BT insecticide, and also between chemical control and release of bracon wasp parasitoid at large larval stage treatments (Figure 3).

Figure 3. Number of seeds per pod at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

The control treatment, showed the highest hundred seeds weight, followed by chemical control and BT treatments. There was no significant difference between the treatments of BT insecticide and release of wasps at large and medium larval stages, in terms of their impact on chickpea hundred seed weight (Figure 4).
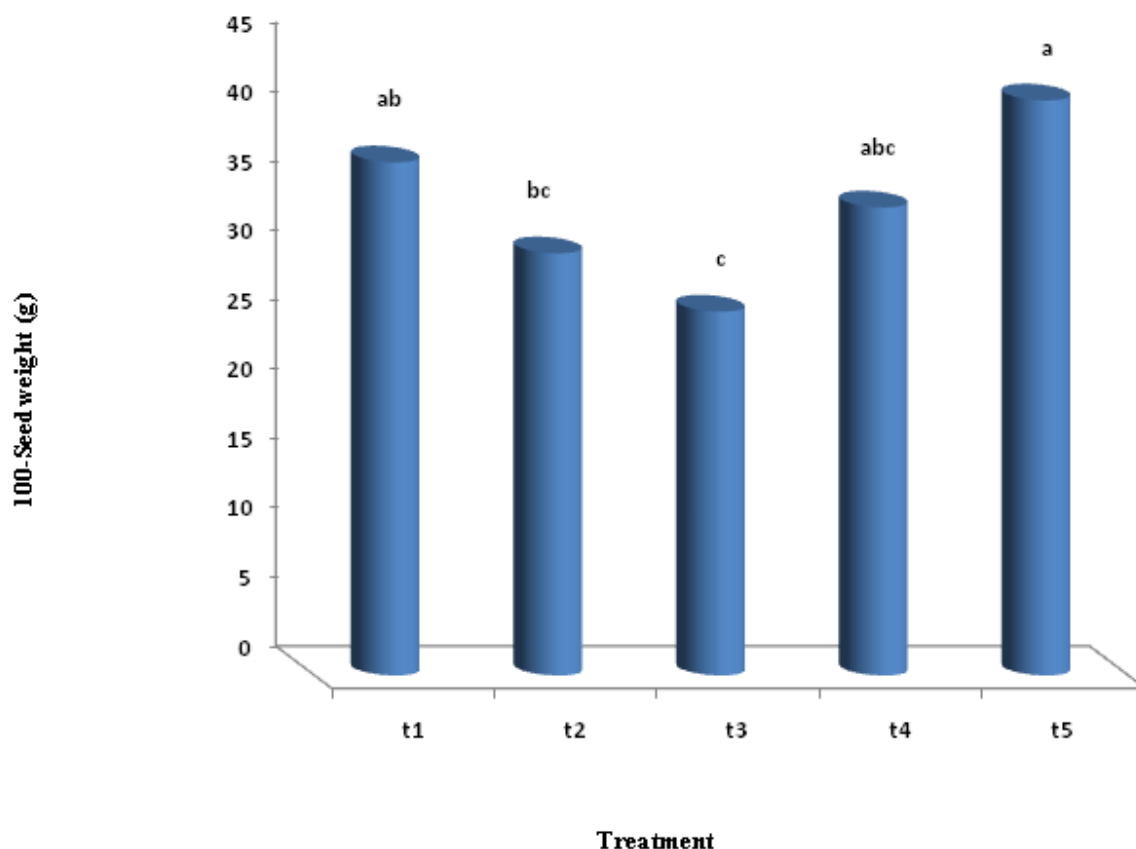

Figure 4. 100-seed weight of chickpea at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

Leaf SPAD value and Plant height
The impact of BT insecticide on leaf SPAD value was not different when compared with the impact of releasing the wasps at large and medium larval stage treatments. Moreover, control and chemical control led to the lowest and the highest value of leaf SPAD, respectively (Figure 5).
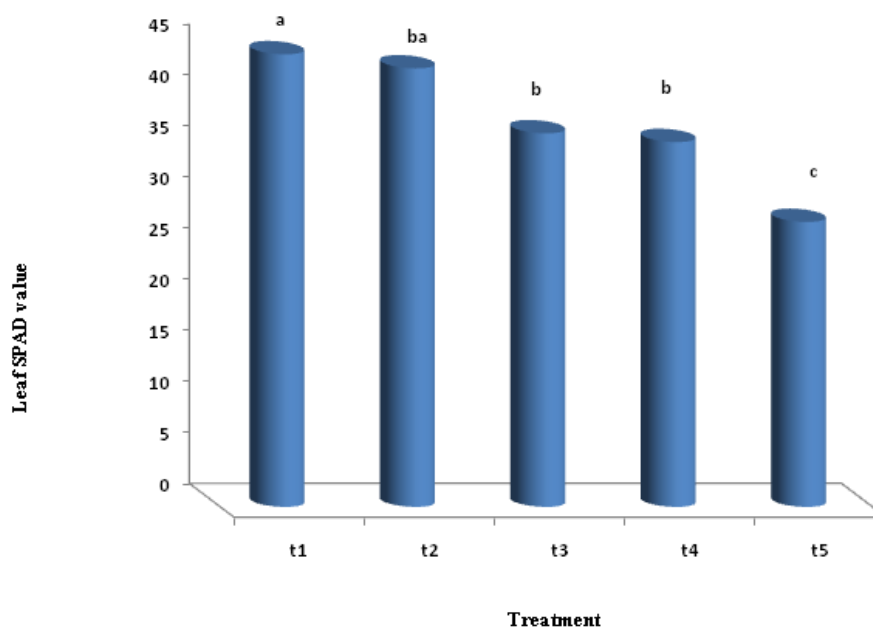
Figure 5. Leaf SPAD value of chickpea at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

Mean comparisons showed no significant difference between the treatments of chemical control and release of bracon wasp's at large larval stage, as well as between the treatments of release of bracon wasps at medium larval stage, BT insecticide, and control, in terms of their impact on chickpea plant height. However, the highest plant height was recorded for chemical control and release of bracon wasp parasitoid at large larval stage treatments, respectively (Figure 6).
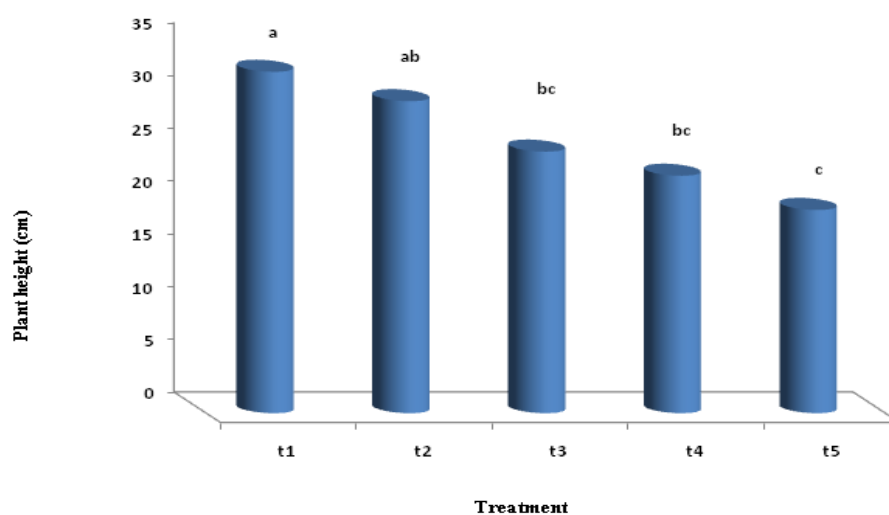

Figure6. Chickpea's plant height at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

Protein content and Seed yield
Results indicated that, control treatment had the highest amount of seed protein content with no significant difference between this treatment and Bt. There were no significant differences between the treatments of chemical control and release of Bracon wasps at medium and large larval stages, for seed protein content (Figure 7).
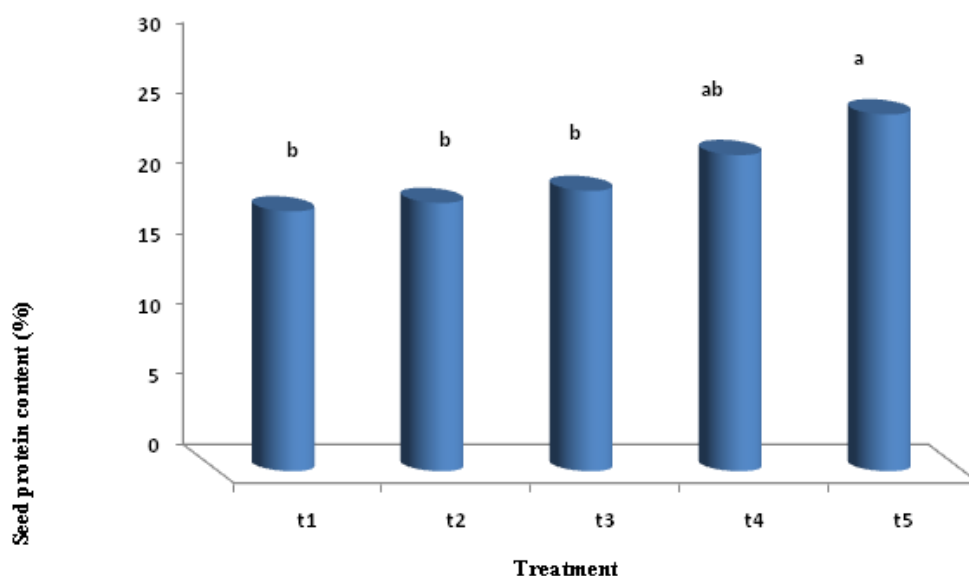

Figure 7. Seed protein content of chickpea at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

Based on the results, maximum chickpea seed yield was obtained from the treatment of chemical control, followed by the releasing Bracon wasp at medium and large larval stages. No control treatment led to the lowest chickpea seed yield. Moreover, BT could not significantly increase this trait as compared with no control treatment (Figure 8).
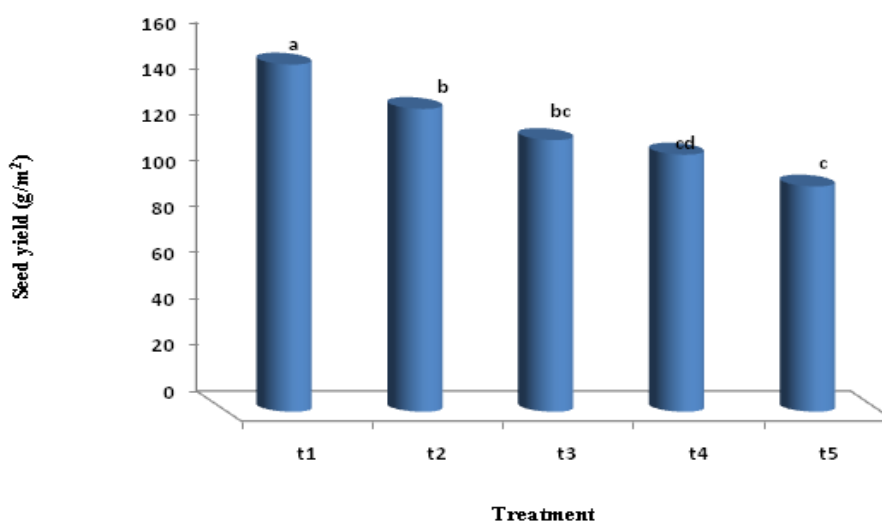

Figure8. Chickpea seed yield at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

Harvest index
Maximum chickpea harvest index was recorded in the plots in which pod borer was not controlled with a significant difference with other treatments. Other treatments including chemical control, BT insecticide, and release of Bracon wasps at large and medium larval stages, didn't show significant differences in terms of the impact on harvest index of chickpea (Figure 9).
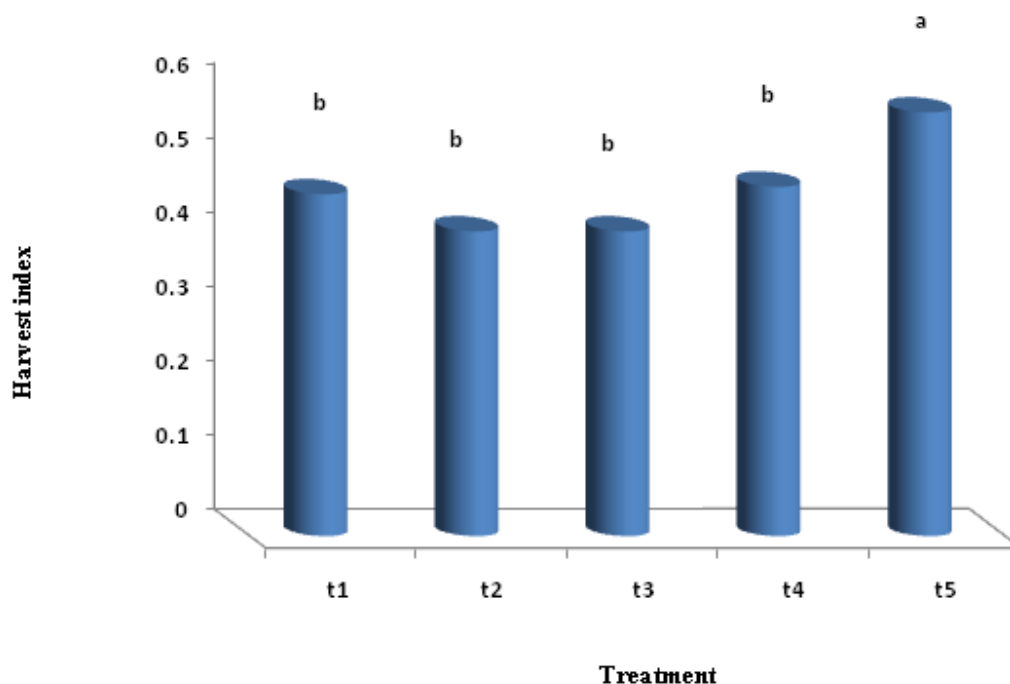

Figure 9. Chickpea harvest index at different control methods. t1: Chemical control, t2: release of bracon wasp parasitoid at large larval stage, t3: Release of bracon wasp parasitoid at medium larval stage, t4: BT insecticide, t5: Control (no control method)

DISCUSSION

The results showed that, chemical control, and release of Bracon wasps at medium and large larval stages, had notable positive impacts on most of the traits including seed yield, the number of pods per plant, and the number of seeds per pod, biological yield, plant height, and leaf SPAD value in chickpea. More effectiveness of chemical control compared with other treatments can be due to high durability and effectiveness of spraying, at all growth stages of pod borer. The frequency of parasitoid releases will impact parasitism of H. armigera and the incidence of plant damage (Li et al., 2006) .However, less effectiveness of BT insecticide can be attributed to low durability of this biological agent during pest growth period (3 to 7 days).The efficacy of Bt, which can be enhanced by incorporation of suitable quantities of acids, salts, oils, adjutants, thuringiensin (exotoxin of Bt) and chemical insecticides, against lepidopteron pests including H. armigera has been demonstrated (Salama, 1984).Application of DiPel 2X and DiPel ES @ 1.6 kg ha-1 and 1.5 l ha-1, respectively, at early stages of crop infestation (1st, 2nd and 3rd instars larval infestation) with at least 2 applications at 7 days interval resulted in significant increases in yield of chickpea as compared to controls (Khalique Ahmed & Khalique, 2012; K Ahmed, Khalique, Malik, & Riley, 1994). Compared with medium larval stage, release of Bracon wasp's at large larval stage, had a higher suppressing impact on pod borer population, likely because of more existing food resources for Bracon wasps in this stage. Maximum hundred seeds weight of chickpea was related to the control treatment; same finding was reported by(kahraryan) based on his investigation on pesticide effect for chickpea pod borer control, who stated that, plots in which Bt insecticide, and Carbonyl and Diflubenzuron pesticides were applied, showed a lower 1000-seeds weight compared to the control, due to lose more flowers and buds. In this respect, the seeds remained on the plant, may use more photo-assimilate, and their weight can increase as a result. He also reported that, Diflubenzuron treatment had a

higher 1000-seed weight, compared to BT insecticide. Control treatment showed the highest harvest index. Although, both seed and biological yields were lower in this treatment compared to others, but the reduction for biological yield was higher, resulted in a higher harvest index. Control treatment also showed the highest protein content. Regarding the role of growth condition after seed filling, we believe that, before seed filling, most of assimilates are consumed for vegetation or flowering, while during seed filling, most of assimilates are designated to this stage. Therefore, a decrease in produced dry matter after pollination, because of pesticide application followed by lose flowers and buds, is likely to play a role in prediction of harvest index. Since, the highest damage of chickpea pod borer was observed for control treatment, we concluded that, the treatment has experienced stress condition caused by the pest, through which the protein content has been increased (Mozaffarian & Sanborn, 2013), in a bio- Ecological study on chickpea pod borer, suggested that the pest has two generations per year, which the damage caused by the first generation is highly important. Damage of the first generation on pods has been estimated to be at least 15 percent. In this study, dominant wasp species was Bracon hebetor, which influenced at least 30 percent of large larvae of the pest. This researcher, in his parallel study on the effect of microbial poisons derived from strains of the bacterium Bacillus thuringiensis on Heliothis larvae, concluded that Bactospin and Delfin are capable to decrease the damage of the pest by 4 percent.

Conclusion
Based on the results shown for all the treatments, despite the increase in the number of pods per plant, number of infected pods was significantly decreased, after applying treatments. In addition, chemical control treatment had the highest impact on chickpea pod borer population, compared with other treatments, which can be caused by applying chemical control at tiny larval stage of the pest and durability of poison effect during pest growth. Due to high cost of chemical control and the problems caused by the use of chemical poisons for environment and human beings, it can be said that, biological control of pests might be the best alternative way to reduce costs and avoid the dangers of using chemical poisons.

Acknowledgement

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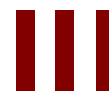
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