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Impact of Green Building Construction on the Environment

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***Abstract*—** This study investigated the impact of construction of green buildings on the environment. In different parts of the study, incentives for construction of green buildings, green building goals, plans for creation of these buildings, comfort indoor quality of green buildings, method of using renewable energy in them to protect the environment were investigated. Finally, it was concluded that in the present world, construction of green buildings and the use of renewable energy seems necessary due to increasing environmental pollution and increasing population growth and scarcity of fossil energy sources.

Keywords— green building, renewable energy, zero energy buildings, LEED technology

I. Introduction

According to statistics of Information Center of Planning and Architecture, total area of the buildings that are built on this planet is about one-sixth of the water areas such as rivers, lakes, seas and oceans. More than a quarter of land under cultivation and forests have been damaged and dried to build new home or factory. Two-thirds of the building materials used in the various buildings caused destruction and loss of incredible amounts of energy from underground sources.

If we want to continue move forward with the same intense speed by which we have had traveled so far, land will not tolerate any human in next few years. This is because limited resources will be finished completely and trace of beautiful forests and oceans will be remained. So in today's world, it seems necessary to use renewable energy instead of fossil energy, especially solar energy, in buildings and residential houses.

The idea of Green Building offers an appropriate solution to our problems. In green buildings, the most important issue is to ensure and provide physical and mental health of human beings. Green buildings can save future of the land and give future generations the opportunity to live together with peace and tranquility.

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II. What is green building?

Green building means a building that would create the least pollution and interference in the environment and it is used for the supply of renewable and clean energy. Green building is a trend which is compatible with the environment and conservation of land over building lifetime. The building itself, its design, construction, operation, maintenance, repair and demolition is consistent with the environment. Construction of green building requires the cooperation of design team members. Architects, engineers and buyers at every stage of green building completion are working to develop the project and complete its classic design and build it based on high level standards in terms of cost, durability and comfort. Although technology has steadily evolved to become a complement for more construction of green building activity, they are designed to reduce the overall impact of the built environment on human health and the environment through:

Beneficial use of water, energy and other resources protect the environment and improve health worker performance
Reducing waste, pollution and environmental degradation

A similar concept is natural building. It is a building with smaller infrastructure and its main purpose is the use of natural ingredients that are native to the area. Other related issues include sustainable design and green architecture. Sustainable design means to meet the building needs of the present generation without compromising the ability of future generations to meet their needs.

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III. The positive effects of green buildings on the environment

Green building activities aimed at reducing the environmental impact of buildings. Therefore, the first law in this regard is that most green building is a building that is not constructed. New building construction often reduces a building's infrastructure. So not to build a building is preferable over construction of green buildings. The second law is that building must be as small as possible. The third law is that it should not help to dispersion (cities tendency to

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irregular distribution). No matter how much green space exist on the roof or how many double glazed windows exist in the building, if you help dispersion in fact you you've failed. Congested areas are preferred to the countryside and green spaces. Buildings are responsible for the huge area of land. According to the National Resource Statistics, 107 million acres of land in the United States are developing. The International Energy Agency in a publication has estimated that existing buildings include 40% of the total energy consumption and 24% of carbon dioxide.

These buildings are outstanding features that provide benefits to the environment by saving and creating a cleaner environment.

The implementation of these features in the environment can be summarized as follows:

-Light paths direct the natural light into the building and reduce the need to the use of electricity.

- Central courtyard direct the natural additional light to the building space.

- The use of lighting and ventilation systems and energy efficient meets environmental standard and it is controlled 24 hours a day or seven days a week to ensure the efficient operation

-Materials used in the buildings are produced from renewable and recycled resources and they will reduce waste materials to save energy

- The use of green roofs, its waters and water collection systems will reduce the building interior and exterior water consumption.

-Implementation of holistic and integrated projects where the building needs of heating is received from the sun to generate electricity while building is cooled naturally and makes freshness. In this case, energy production cost will be saved.

- A green house is a small ecosystem that refines the sewage

- Roofs are covered with special plants of dry climates and heat-resistant plant species. This reduces heat exchange in the impermeable roof area. Therefore, the heat reflected back into the atmosphere is reduced and the light pollution and energy production also will be reduced.

- In this project, a series of wind turbines produce energy.

IV. LEED Technology (Leadership in Energy and Environmental Design)

Green buildings are also known as sustainable buildings are among structures that allow optimal utilization of precious natural resources such as water, wind, solar energy, etc. along with providing effective and recyclable materials for building. These buildings experienced remarkable progress in design and technology in recent years. This caused reduction of environmental pollution and subsequently healthier environment inside and outside buildings. The contamination is caused by demolition and re-construction of building or it has occurred due to air and soil quality or non-clean energy consumption. With expansion of green building construction in public and private sectors and interest of industrialists and construction professionals, the need to a regular program is inevitable. To this end, the United State Green Building Council (USGBC) designed a plan to use principles of operation of green buildings in the whole world. This plan called LEED that is abbreviation for Leadership in Energy and Environmental Design. This plan means management of energy and environmental design. It is based on the principle of energy and the environment. This design is actually an essential factor of balance between important and effective functions of environment. Project teams (owners, developers, architects and contractors) with regard to the principles of this program can be a powerful tool for guiding and managing economic and physical guidelines in order to help green projects goals.

V. HOW DOES LEED work?

LEED works based on 5 environmental principles:
1 - Environmentally friendly sites. 2 - Water efficiency (Water conservation). 3 - Energy and atmosphere. 4 – Maintenance of materials and resources. 5 – Internal quality of building in terms of environmental issues

If a project is designed according to these 5 factors or in other words to be complying with the LEED criteria, then it will provide an integrated result and may obtain silver, gold or platinum certificate. This will indicate the amount of attention to the environmentally friendly principles in the certifications:

1- Consideration of these factors in environmentally friendly sites during site designing causes some impacts on this sector.
(A)Building site
(B)Area designing based on natural and agricultural environments
(C) The use of vacant lots between buildings and lands which contaminated by previous applications
(D) Reduction of need to use cars
(E) Optimization of local area: Management and control of surface water
(F)Reduction of pollution

2- Water conservation
(A) Reduction of the amount of water needed for the building and people (water saving)
(B) not to use potable water for irrigation and washing.
(C) The use of new technologies for wastewater treatment
(D) the protection of water quality and drinking water, rivers, streams and lakes

3 -Energy and Atmosphere
A: Management of impacts on the atmosphere and energy as far as possible for reduction of energy consumption
(B) The use of renewable energies.
(C) Periodic maintenance of buildings based on principles
(D) Removing halons and carbon
(E) Preventing depreciation of ozone layer

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4-Materials and resources
(A) reusing the existing buildings
(B) reducing the amount of consumed materials
(C) The use of local, regional and renewable materials
(D) The proper use and replacement of wood resources
(E) Reduction of waste and its management

5-Internal building quality in terms of environmental issues

(A) The removal or reduction of pollution sources within the building
(B) Air polluters controlling
(C) Thermal studies and prevention of heat loss.
(D) Air quality control
(E) The proper and optimal use of light

In terms of environmental benefits, construction and building has a large negative impact on forests, grasslands, plants, animals and agriculture ecosystems. ☒
With proper selection and general layout of buildings in suitable locations, it will be possible to prevent excessive expansion of urban areas in major cities as a new problem. Renovation of existing buildings, the use of wastelands between buildings and the grounds contaminated in the past because of machine method of life can prevent excessive growth of cities.

Economic benefits of reduction of the cost of operations:

The cost of energy and water in buildings constructed by LEED technology has been reduced considerably compared to old buildings. This amount can be handling over a period of time to obtain the initial cost of investments. Buildings built with this technology can lead to the development of future projects. Renovation of existing buildings and infrastructure can reduce construction costs. In this system, some capabilities can be used in a project to support another project. With shrinking of some equipment such as chillers, over consumption can be prevented. Occupation of land, less water resources infrastructure to refine, reduce operating costs, reduce the costs imposed by the environment, are all important factors in improving economic performance. Increasing of desired environmental benefits and value can be considered as a ground for valuable profits in society. Improving efficiency in production caused increased profitability of public health and also reduced health care costs. This large planning raises the real value of the organization and number of applicants will be increased. Green buildings designed with the correct operation will be sold or rented more quickly due to more convenience and greater physical facilities and economic benefits provided for users. Imagine a future world where green design is growing and changing while this kind of design is a factor that transfers environmental friendship to the next generation.

VI. Zero-Energy Building

High energy consumption and consequently increasing population and increasing urbanization has led to find methods to access new energy resources to increase energy efficiency. Moreover, it is attempted to provide comprehensive environmental rules applicable for energy consumption, reduce emissions from fossil fuel consumption, reduce greenhouse gas emissions resulting from the energy sector and reduce negative impacts on the economy. So that in the twenty-year perspective on the general policies of the Islamic Republic of Iran and other energy sources, diversify of the country energy sources and its use in compliance with environmental issues and efforts to increase the share of renewable energy is mentioned. Despite huge resources oil and gas in Iran, it is necessary to pay more attention to renewable energy sources such as wind movers, solar, biomass, geothermal, hydro, nuclear, hydrogen, fuel cells, biogas, etc. One of the most important ways to reduce consumption of fossil energy is construction of zero energy buildings.
Zero energy buildings are defined as buildings that their annual energy consumption is zero and do not produce carbon pollutants. In today's world, due to the limited fossil fuel resources, buildings, industries and other organizations are turned to the use of other energy sources such as solar, wind, and hydro-biological resources.
The idea and principle of zero net energy consumption has attracted too many attentions because using of a renewable energy is a strategy for the removal of pollutants and greenhouse gases. Today, "zero energy" designs have a special popularity due to rising costs of fossil fuels and their harmful effects on the environment and climate, and their disrupting effect on the ecological balance. This structure can be independent of the power supply network. Thus, energy is supplied locally and through a combination of technologies to produce renewable energies such as solar, wind and bio-fuels energy. However, by the use of specific technologies for ultra-efficient lighting systems and heating and cooling system, attempts are made for less and less energy consumption. In other words, in a zero energy building, before producing clean energy to optimize the energy consumption in different parts of the building, it is focused on the clever use of renewable technologies, the balance between production and consumption of energy. Currently, administrative and residential buildings account for about 40 percent of the country's fossil fuels consumption. Although zero energy buildings, even in developed countries today are very rare, they have been growing and attracted too much attention due to being independent of fossil fuels and helping to reduce carbon emissions.
Green building refers to a collection of buildings that are committed to environmental conservation over the life time of a building from design to construction and operation or reconstruction. In these environmentally friendly buildings, the use of renewable energy is in priority. Moreover, its energy consumption is negligible and its materials are also assessed in terms of environmental issues. The ultimate goal

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of green building is efficient use of resources and reducing the negative impact of buildings on the environment. Zero energy buildings is one of the key objectives to achieve green buildings and reduce pollutant and greenhouse gas emissions during the use of buildings. However they cannot be considered as "green" in all areas such as waste reduction or using recyclable materials. One of the main goals of green building and zero energy buildings to reduce energy consumption for heating, cooling and electricity as well as increasing the energy efficiency of buildings. To reduce energy consumption and construct green building, building designers should reduce waste of energy in buildings. Consequently, available strategy is the use of high-performance windows and insulation of walls, roofs and floors in the building.

Green building strategy that can be used by power engineers is building design based on the view of the use of solar energy that is usually performed in energy efficient buildings. In these buildings, positioning of windows, walls, porch, canopy and trees should be oriented so that cause shade in summer and the maximum solar gain in winter. In addition, window proper location can increase the amount of day lighting and reduce energy consumption of electric lighting during the day. Among useful strategies in this section are the use of technology of active solar, passive solar, photovoltaic and roof garden.

The use of integrated building management system (BMS) is another approach that plays a significant role in these buildings. This system controls the various parts of the building including heating and cooling installations, lighting, fire alarm, building doors ,etc. which enables the management and reduction of energy consumption. For example, the building equipped with precise temperature and humidity controller, adjustable operating time and other parameters can save up to 20% of energy consumption.

VII .CONCLUSION

According to the results of the study, it can be found green building has many advantages including: Increased energy efficiency (reduced heating and cooling costs of the building, temperature regulation), reduced effect of heat island, promoting the Mental state of residents, protection of buildings, increased property value, management of water storms, reduced emission of greenhouse gases, protection of the building, creating local jobs, reduced noise. According to ever increasing environmental pollution caused by rapid population growth and consumption of fossil fuels, construction of green buildings in the current world seems necessary to protect the environment Abbreviations and Acronyms

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A Study of the Application of the Parasitoid Wasp, *Trichogramma Embryophagum*, in Controlling the Carob Moth in Zirtang District of Kunani Town of Iran

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Abstract: The carob moth, *Ectomyelois Ceratoniae*, is the most important pest of pomegranate fruit in Iran and on average, renders 30-40 percent of pomegranate crops useless and spoiled when still on the tree or in the warehouse. In the process of implementing the integrated controlof carob moth program, the significant activity of parasitoid wasp, *Trichogramma Embryophagum*, was considered in Zirtang district of Kouhdash city of Iran. In order to increase the degree of parasitism, in autumn 2012, the eggs containing the parasite were collected and delivered to laboratory of Agriculture Jihad. The wasp species of the region was identified and reproduced, thenthey were used in the form of tricho cards. In the garden under experiment with the area of 7000 sq. m. which had 400 trees, the releasing was carried out and its results was examined in comparison to the control plot. Since June 22, 100 tricho cards containing 2100 eggs with parasite of *Ephestia* were installed on every other tree every 10 days. Totally, there were 10 releasing and the control was implemented from June 22 till Nov. 21, 2012 through three tests. By examination of population changes of carob moth, examination of changes in eggs with parasites, and examination of the number of the contaminated and wormy fruits in a T test plan, the difference of the crops amount of the plot in which releasing was carried out and control plot on Nov. 21 were compared and calculated. And the difference was significant and the contamination of the fruits decreased 55% as a result of releasing.

Key words:wasp, parasitoid, moth, caro

Introduction

Pomegranate is one of the valuable horticultural crops of Iran which is for export, and because of its quality and not using pesticides, has attracted the attention of international markets, especially in recent years. Carob moth, *EctomyeloisCeratoniae*, is currently the most important pest of pomegranate in Iran. This pest is multi-generational and was observed in Kashmar town gardens in 1970 for the first time and Pazooki identified it. Later, it was named as carob moth by Sharifi (Khodakaram Tafti, 1995, Zare & Shahrokhi, 1995). This insect became revolting since 1981 in most pomegranate gardens of the country and in some years, up to 80% of the pomegranate crops were rendered useless and spoiled while on the tree or in the warehouse (Shakeri, 2004). This pest is polyphagous and acts on other crops like figand harms them (Mehrnejad, 2002, Shakeri, 1993). Because of the biological characteristics of this pest that practically do not allow using pesticides, most of the control methods recommended for this pest in order to decrease the damage, are based on farming and mechanical methods, such as removing the stamen, covering the pomegranate calyxin mud (Rajabi & Farzaneh, 1998), and collecting and destroying all the contaminated pomegranates during farming season and winter. Application of *Trichogramma* wasps is in the winter. Its application has been operationalized in some regions. Given the results of the researches and conducted studies to control carob moth, currently, none of the typical methods can alone control this pest. Logical and effective control of carob moth is only possible through an integrated control. The degree of parasitoid of the *Trichogramma Embryophagum* wasp on carob moth was reported on average up to 17% (Mirnejad, 2011). The degree of natural parasitism of this parasitoid in

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Pishva Varamin town is reported as 11% on average (Nouri Zadeh, 2011). Releasing parasitoid at the time of appearance of carob moth since end of June was carried out in the form of distributing tricho cards in the garden. Bayat Asadi also began releasing in Yazd city of Iran at the end of June. As Shojaee recommended, the efficiency of the aforementioned parasite is higher in gardens which follow improved farming (Bayati, 2010). Tricho card containing 0.01 g. egg with parasite were deployed on rice shrubs by Bayat Asadi with the spacing of 10 meters (Bayati, 2009). From among the different methods of releasing which has been tested by kelozen mixed with sawdust and waterlogging and inoculation method, using tricho card on pomegranate has been the most proper method (Shojaee, 2011). Wagenberg has presented satisfying statistics regarding the application of *Trichogramma* for controlling carob moth around the world (Wagenberg, 1991).

Material and Methods

In order to increase the population of the aforementioned wasp, some samples of the eggs containing the parasite were collected and delivered to the *Trichogramma* keeping laboratory. Then, the reproduced wasp species was released in the Zirtang district in pomegranate gardens. In Feb. and March of 2013, 2 plots of pomegranate in Zirtang district, each with the area of 7000 sq. m. were selected. Releasing the parasite in plot one was carried out and the plot two was considered as the control plot. The number of trees in each plot was 400. The gardens had 10-year-old trees which were healthy and productive and their height was 3 meters on average. The trees' spacing on the lines was 3.5 meters and the distance between the lines was 4 meters. All the agronomic principles including irrigation, pruning, weeding and spreading manure were followed in a proper and uniform manner in theplots, especially the weeds around the trees were removed every 20 days. Since about May 21, the releasing was carried out every 10 days. Each time one tricho card havingeggs containing parasites of flour moth, *Ephestia*through*Trichogramma* parasitoid wasp was attached to the inner branches which were at least 2 meters above the ground, using a tape. Each card contained about 2100 eggs with parasites of *E.Kuehniella*. Tricho cards were installed on every other tree each time. First, in the first line, the trees 1, 3, 5, etc. and in the line two, the trees 2. 4, 6, etc. the releasing was carried out, and the next time (10 days later) the procedure was the reverse, in such a way that the releasing was not carried out on the tree on which it was done in the first time, but it was carried out on the tree next to it. So during two times of releasing, all the trees had tricho cards. Tricho card installing was performed since Apr. 20 till July 22-27 every 10 days. Altogether, 10 releasing (tricho card installing) was carried out. In samplings for determining the population of adult insects, light trap was used. A light trap was placed in the middle of the plot under experiment and another one in the middle of the control plot, the number of moths in each trap were counted once a week and they were removed. In each plot consisting 400 trees, starting from tree number 1, one tree was chosen out of every 10 trees. 6 pomegranate fruits were collected from each tree, 2 from upper parts, 2 from middle parts and 2 from lower parts. Totally, 120 pomegranate fruits in each sampling were collected. In the second time, the procedure began from the second tree and 1 tree was selected out of 10 trees, and in the third time, it was started from tree number three and so on. In the laboratory, eggs with parasites and healthy eggs were counted in the calyx. In order to count the healthy and contaminated pomegranates, samples were gathered from Sep. 22 till Nov. 20, every 10 days from control plot and plot under experiment.

Results and Discussion

The releasing was carried out since about May 21, every 10 days. Since May 21 till Nov. 22, 120 pomegranate fruits were harvested randomly from control garden and the garden in which releasing was carried out every week, and the number of eggs with parasite and healthy eggs was counted in the laboratory. Counting the number of healthy and contaminated pomegranate fruits to carob moth in control plot and plot in which releasing was carried out, was performed since Aug. 20 till about Nov. 20. The mean variance analysis is presented in tables 3 and 4. According to the samplings performed through light traps in control plot and plot in which releasing was carried out, each with the area of 7000 sq. m., appearance of the moth began from about May 20 and continued until September. The results indicated that the population of the moth reached a peak in 2012. Table 1 shows the population changes of carob moth in plots under experiment. The population of adult insects of carob moth considerably decreased due to releasing parasitoid wasp. Al Maliky(1986) reported that the parasitism begins with a degree of 10% in April and reaches 35% at the end of the season. Mehrnejad (2002) reported the degree of parasitism of larva stage of carob moth in Palestine on Acacia by *C. Saturata* as about 33.4%. The degree of parasitism maximizes at the end of farming season. The activity of parasitoids in pomegranates fallen on the ground is lower, and it appears that this useful insects prefer to put parasites in larvae inside pomegranates on the tree. Farzaneh (1975) believes that the best way to control this pest is to decrease its winter storage, which is in the form of larva inside the remaining pomegranates in the garden. According to Shakeri (1993), in addition to gathering contaminated pomegranates in autumn, gathering pomegranates contaminated to first generation of larvae in the following year and destroying them is essential; thus, one of the ways for controlling the pest is gathering and destroying contaminated pomegranates in autumn and winter as well as in farming season. Carob moth in Zirtang district has three complete generation and 1 incomplete generation in a year. In the control plot, the population of the carob moths reached a peak 4 times. Moths have overlapping generation and their population is more than previous peak, while, in the plot in which releasing was carried out, the peaks were rather

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unknown. In table 1, the number of counted moths in the control plot and plot under experiment since May 21 till July 21, do not have significant difference.

Table 1. Population changes of carob moth in plots under experiment in 2012

Number of moths in the light trap in plot after releasing	Number of moths in the light trap in control plot	week	Sampling date
6	5	Week 1	
9	11	Week 2	May 21- June 20
13	12	Week 3	
15	16	Week 4	
18	21	Week 1	June 21- July 21
19	20	Week 2	
15	17	Week 3	
12	15	Week 4	
7	12	Week 1	July 22- August 21
27	32	Week 2	
20	27	Week 3	
10	24	Week 4	
5	25	Week 1	Aug. 22- Sep. 21
16	32	Week 2	
11	37	Week 3	
8	31	Week 4	
9	24	Week 1	Sep. 22- Oct. 21
5	21	Week 2	
6	11	Week 3	
12	23	Week 4	
13	26	Week 1	Oct. 22- Nov. 20
5	11	Week 2	
3	10	Week 3	
4	8	Week 4	
11.8	20.47		Average

Table 2. Changes in the number of eggs with parasite during sampling in 2012

Number of eggs with parasite in plot after releasing	Number of eggs with parasite in control plot	week	Sampling date
		Week 1	
		Week 2	May 21- June 20
7	3	Week 3	
15	4	Week 4	
25	7	Week 1	June 21- July 21
31	12	Week 2	
36	13	Week 3	
49	15	Week 4	
59	19	Week 1	July 22- August 21
73	22	Week 2	

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79	24	Week 3	
82	27	Week 4	
88	29	Week 1	Aug. 22- Sep. 21
66	30	Week 2	
66	28	Week 3	
58	25	Week 4	
72	25	Week 1	Sep. 22- Oct. 21
66	23	Week 2	
66	22	Week 3	
58	21	Week 4	
55	18	Week 1	Oct. 22- Nov. 20
49	16	Week 2	
36	14	Week 3	
13	11	Week 4	
41.67	17		average

The average difference of moths during the season in comparison to the difference of the number of eggs with parasite and contaminated pomegranates in two plots is not considerable or less. The results from conducting this experiment shows 55% increase in crops as estimated and observed.

Table 3. Mean of treatments

Contamination percentage	
20.2	Released parasite
82.8	control

Table 4. Variance analysis

F	MS variance	Degree of freedom	Source of changes
1568.153	12167.230	1	T treatment
	59.453	18	C error
		19	total

CV=11.88% the difference released and parasite treatment is totally significant

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Influence of Agar Concentration and Liquid Medium on In vitro Propagation of Stevia (*Stevia rebaudiana* L.)

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Abstract

Stevia is an important and economical herb that contains natural and non-caloric sweetener. This substance can be alternative to artificial sweeteners such as Aspartame and Sodium Saccharin used in the food and drug industries. The most important characteristic of this plant is their suitableness to use for people with diabetes. However, at the first step low viability and low seed germination, limit their extensive cultivation. One of the effective methods to produce this plant is in vitro technology that solves the problem of large scale production of this plant. The purpose of this investigation is the study of effect of different concentration of agar on the in vitro micro propagation of Stevia plant. For this purpose, MS culture medium with different concentration of agar (0.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12 g/l) with two explants (Shoot tip and Node) were used. After 4 weeks shoot length, number of leaf, number of root and root length were evaluated. The analysis of collected data statically (ANOVA) showed that there is significant difference between studied parameters and different concentration agar. The obtained results demonstrated that shoot length, number of leaf, numbere of root and root length in shoot tip explants have increased above 2.0, 2.0, 5.0 and 5.0 folds responding at the Liquid medium (without agar).

Keywords: Agar, Explant, Micro propagation, Stevia

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is the most valuable tropical medicinal plant, belongs to the family of Asteraceae. It is a natural and sweet herb that is native of northeastern of Paraguay [3]. Stevia is the new emerging alternative source of calorie free sweetener having no carbohydrate and fat. It is 20 to 30 times sweet than cane and beet sugar, highly nutritious, delicious, non-toxic and non-additive sugar [11]. It also enhances the flavour, helpful in digestion, weight reduction, anti oxidant, prevents dental caries and having antimicrobial and anti plaque properties, increases mental alertness, increase energy levels but does not affect the blood sugar level, therefore key-source sweetener for diabetic world [6]. Besides, Stevia can be used in hypertension, hypoglycemic, helpful in skin toning and healing, tobacco and alcohol cravings and a tonic for pancreas. It can also be used as alternative source of sugar for food confectioneries, bakeries, fruit, juices, jams, biscuits, chocolates vegetables and other food stuffs [6].

The recent researches along with future prospective of this new emerging medicinal plant. Stevia is a valuable medicinal plant species and it is being used for the treatment of diabetes. There is a high demand for raw material of this medicinal herb due to ever increasing diabetes disorder among the population [7].

Stevia is a herb that is used extensively in various areas of the world as a non-caloric sugar substitute. Due to its huge applications in food, drugs and pharmaceutical industries, it is now commercially cultivated in many countries of the world viz., Brazil, Paraguay, Uruguay, Central America, Thailand, China and Japan [10].

Currently, stevia is being propagated by stem cuttings. Low seed germination percentage is a major limiting factor for large scale cultivation of stevia plant species for commercial usage. Further vegetative propagation is also limited by the less number of individuals obtained from single plant. Therefore, a suitable alternative method for large scale plant production within a short period is the use of in vitro culture technology [12].

Agar is used for hardening media in tissue culture. General, it is used of the different concentration. Interaction between in vitro raised plantlets with the gelling agent in culture medium is a dynamic process and the changes in gel consistency affect the regeneration of plants or tissues [8]. Traditionally, agar (0.6-0.8%) is added to the culture medium to increase its viscosity. As a result of which plant tissues and organs remain above the surface of the nutrient medium [2]. Increasing agar strength beyond a critical limit has been demonstrated to inhibit organogenesis and shoot

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growth and reduce the water availability to the cultures [9]. Recent reports have suggested that low concentration of agar provides a poorly gelled medium that facilitates adequate contact between the plant tissue and the medium and better diffusion of medium constituents, resulting in better growth and their subsequent rooting [1]. Methods of in vitro propagation in liquid medium have also been attempted where agar was completely omitted from the medium. By using liquid medium instead of gelled medium, propagation is accelerated, culture transfer frequencies may be decreased, labor is less intensive and cost of production is reduced [4]. The present studies deal with the improvement of protocol by manipulating agar concentration in the medium and by using liquid culture system.

Materials and methods

In this investigation were studied effective factors on micro propagation of stevia such as explants type and different concentrations of agar from achieving efficient protocol.

Plant material

Stevia rebaudiana Bertoni plants were procured from Agriculture Biotechnology Research Institute of Iran. In this experiment, shoot tip and node segments were used as explants.

Explants sterilization

The shoot tip and node explants were washed in tap water and gently rinsed with 20% (v/v) extra and surface sterilized in 0.1% sodium hypochlorite solution for 10 min and then rinsed with five changes of sterile distilled water.

Culture establishment

In order to different concentrations of agar with two explants were used. The culture medium consisted of MS [5] salts, vitamins, 3% (w/v) sucrose and the pH of the media was adjusted to 5.6 with 0.1 N NaOH or HCl before adding of agar and autoclaved at 121°C for 15 min .After surface sterilized shoot tip and node explants were cultured on MS medium completed with different concentrations of agar (0.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12 g/l) for proliferation. The cultures were incubated at 24±2 ◦C under 16/8 h (light/dark cycle) photoperiod (60 µE m−2 s−1) and irradiance provided by cool-white fluorescent tubes. The shoot cultures grown on both agars gelled and liquid media were assessed and compared for their in vitro growth in terms of various growth parameters like shoot length, number of leaves per cluster and number root. To find out the effect of different concentrations of agar on in vitro rooting, its concentration was varied from 0.0 to 1.2% (w/v) in standard rooting medium. In case of 0.2% agar and medium without it (liquid medium), filter paper bridges were used as support matrix. Agar at 0.8% concentration was treated as control.

Statistical analysis

Experiments were done in factorial based on completely randomized design (CRD) with 3 replications and observations were recorded after the 4 weeks. The analysis of variance (ANOVA) was performed using SAS program. The differences among means were determined by Dunkan Test at 1% significant level.

Results and Discussion

The impact of different concentrations of agar (0.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12 g/l) were examined on growth vigor and rooting capacity of in vitro propagated plantlets derived from apical shoot and single node cultures. The statistical analysis of variation for shoot growth, leaf number, rooting rate and roots length per plant showed significant differences for all characters (Table 1).

Shoot length

Interactive source variation of Agar × explants showed significance at $p<0.01$ for length of in vitro produced plantlets (Table 1). Results of shoot length were illustrated on figure 1, The maximum shoot length were derived on the liquid medium (without agar) from shoot tip explants. On the contrary, increasing the concentration of agar to 1.2% in the medium caused significant reduction in overall growth of shoot cultures of the stevia. The differences between two explants type could be due to substantial vigor of shoot tip against node explants leading to develop strong plantlets, whilst high concentration of agar (1.2%) prevented adsorb required water and required nutrient elements.

Table 1: Analysis of variance (ANOVA) for micro propagation parameters of *Stevia rebaudiana*

Source of variance	Degrees of freedom	Mean square			
		Shoot length	Leaf of number	Root of number	Root length

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Agar	6	1760/19**	56/12**	40/78**	603/70**
Explant	1	1208/68**	17/73**	11/43**	456/88**
Agar × Explant	6	57/29**	5/65**	2/83**	33/04**
Error	28	32/92	1/91	0/73	16/12
٪CV		18/7	11/6	8/8	14/5

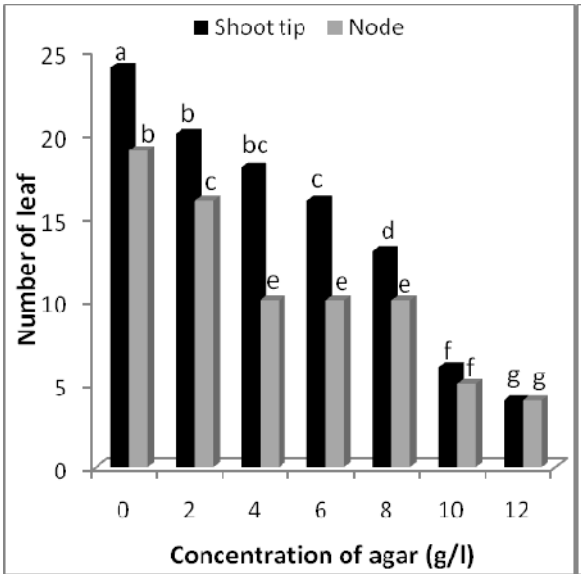

Fig. 1. Effect of different concentration of agar on the shoot length

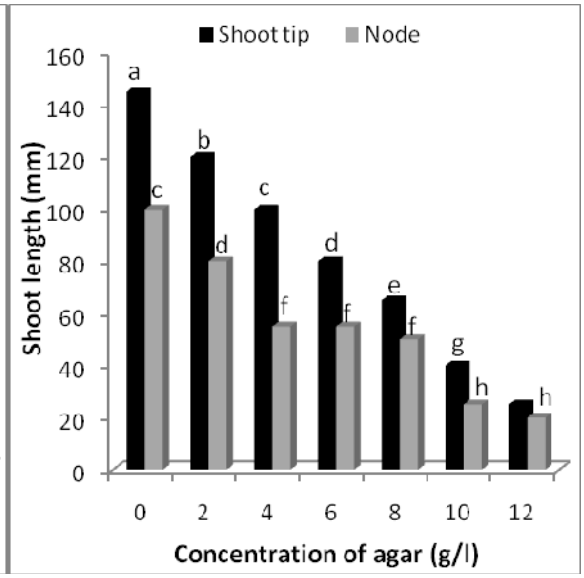

Fig. 2. Effect of different concentration of agar on the number of leaf

Number of leaf per plantlet

Apart from shoot length higher number of leaves in each plantlet is considered a useful trail from multiplying by in vitro methods. The effect of different concentrations of agar on production leaf in the explants typ is more similar to variations observed for shoot length. So that, the number of leaf decreased with increasing the agar concentration in explants typ, possibly due to reduced water availability. The maximum highest Leaf number pre plantlet were recorded on the liquid medium (figure 2).

Nu

Dif cant effect on root length and number of root. So that, increasing the concentration of agar in the medium were caused significant reduction number of root. The maximum of number of root (2.33) was obtained in standard condition (8 g/L agar) from shoot tip explants. But, the best respond root induction (9.8) was observed at liquid media (without agar) from shoot tip explants (figure 3).

Root length

The effect of different concentration of agar on root length process of explants is more similar to variations observed in number of root and among all the treatments, the highest root length (5.367 cm) was obtained from shoot tip explants in the liquid media (figure 4).

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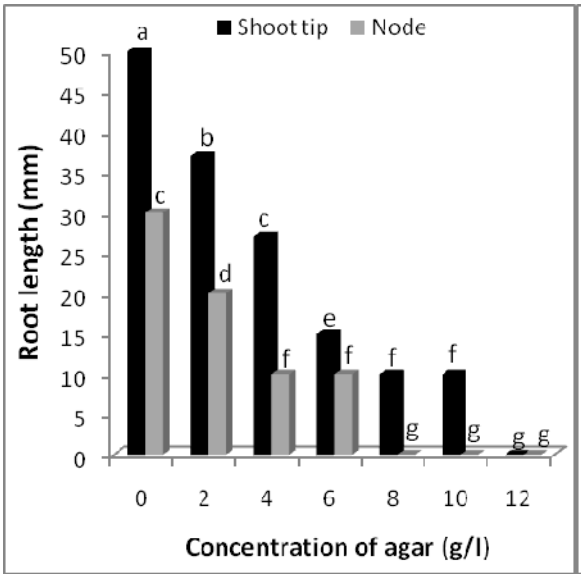

Fig. 3. Effect of different concentration of agar on the number of root

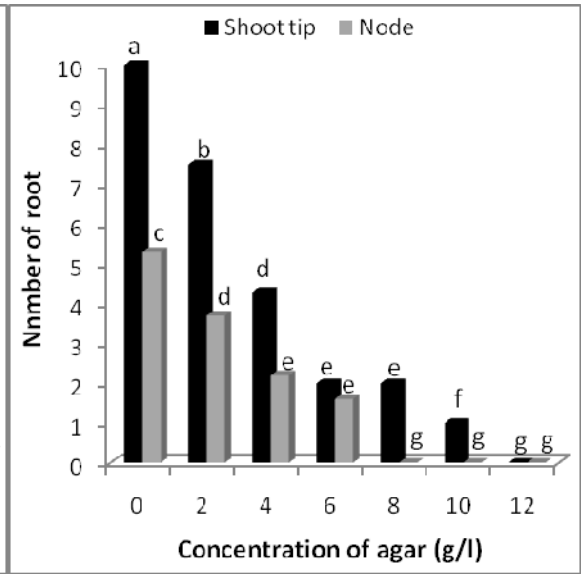

Fig. 4. Effect of different concentration of agar on the root length

Conclusion

Agar is used often for hardening media. Generally it was used at different concentrations. Low levels of agar and liquid culture medium because of faster uptake of nutritional requirements and better absorption of water by plants have been reported to promote shoot proliferation in several culture systems(8,9). In other hand, increasing the concentration of agar in the medium caused significant reduction in overall growth of shoot cultures. Studies on the *Stevia rebaudiana* showed that reduction in growth parameters were recorded on the medium gelled with higher concentrations of agar. Lowering of agar concentration and use of liquid media that gave better micro propagation protocol of *Stevia rebaudiana* is a suitable alternative.

Acknowledgements

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A Study of the Existence of Various Biotypes of Corn Common Smut Agent, *Ustilago Maydis*, in Lorestan Province of Iran

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Abstract:
In order to study the existence of the biotypes of corn common smut in Lorestan province of Iran, the isolates that had the highest percentages of germination were selected from among the isolates collected from each township; then, these isolates were inoculated to selected corn lines after appearance of maize clusters. The results of the present study revealed that the percentage and the severity of infection of isolates were different in various lines. According to these results, it can be argued that K17/2-3 and TV926 lines had the lowest and the highest resistance to used isolates, respectively. The highest and the lowest percentages of pathogenicity belonged to isolate number 1 with %95/3, and isolates number 8 and 43 with 50%, respectively. Isolates were divided in two groups for biotypes identification based on percentage and infection density so isolates 6, 7, 8, 9 were placed is same group.

Key word: maize, line, pathogenicity, genetic similarity, gall, severity

Introduction

Ustilago maydis (syn. *U. Zeae ung*.) is the agent of corn common smut disease. The disease was reported from Europe in 1754 for the first time and then it was seen in United state of America in 1823 (Alizade et al., 1999; Chogan & Zamani, 2000). In Iran, it was reported for the first time from a small field in Semnan City in 1981 and then it was seen in Gorgan and Varamin Cities in 1983 (Nekoyi & Sharif Naby, 2000). High reproduction and relatively long durability of teliospores along with high variability in life manner and special signs that it causes in plants are among distinct features of this fungus (Snetselaar & Mims, 1992; Estakhr & Zamani, 2002). Damage of corn common smut shows itself by reduction of product and formation of galls on each aerial part of plant such as cluster, spike, stem and leaves.

Reduction of product caused by this disease ranges from a slightly damage to 100% or more in different areas. In Iran, there is no exact statistics about dispersion and severity of damage caused by the disease, but some researchers have estimated that it is about 10% of product (Banuett & Herskowitz, 1982; Banuett & Herskowitz, 1996; Batra & sharma, 1968; Martin et al., 1988; Pajohande et al., 2002). Chresteiens et al. (1963) believed that all corn varieties and lines that are infected by artificial inoculation, become highly sensitive to disease, which is due to the destruction of physical resistance of tissues and stems thickness that prevents disease agent inoculum to reach meristemic tissue. He also believed that this fungus has high genetic diversity and because of heterothallic nature of the fungus, it has many biotypes in nature and constantly produces new biotypes as a result of hybridization and mutation in haploid and diploid stages. Damage caused by this disease in an area is variable due to properties such as race of pathogen, oldness of disease in the area, host sensitivity, agricultural practices and amount of consumed fertilizers (Johnson & Christensen, 1935; Hooker, 1956; Martine et al., 1975; Christense, 1963; Ullstrup, 1978; Tajbakhsh, 1982; Martin et al., 1988; Thakur, 1989; Valverde et al., 2000). There are different methods to inoculate and infect corn with corn common smut pathogen. Comprehensive studies were carried out by Pope and McCarter titled evaluating different methods for infection of maize, during 1989 to 1991. The purpose of these studies was to achieve a reliable method for formation of galls and recognizing the resistance mechanisms. Methods such as silk cutting, wood cutting and wood injection for infection of corn to common smut are other methods of Pope and McCarter (1992b). In these methods, after the appearance of tassels and before they dry, 105 mm of sporidium suspension in each millimeter was injected to the bottom of maize husk and the same injection was administered to the bottom of the

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maize that 1/5 cm of them had been cut. The result was the formation of galls in %97 of inoculation cases (Pope & McCarter, 1992a). Baddueyt et al. (2006) conducted comprehensive studied to survey various biotypes of *U. maydis* in Anoya and Batazake in Egypt and biotypes A, B, and C were identified among *U. maydis* samples. According to given results, biotype A was more destructive than biotypes B and C. Studies were done by Sinskey et al. (2006) to recognize the possibility of *U. maydis* biotypes in India and eventually, 27 biotypes of *U. maydis* were proved to be existing. According to current evidence, D. S. of various *U. maydis* biotypes was different. Bruce et al. (2006) carried out studies to determine corn common smut biotypes in Canada; they proved 6 biotypes of the fungus are existing. According to the findings, biotype A, as the most destructive one in Appodomia, caused %98 of infection in line K17. According to Warfied et al. (2006), isolates of fungus of corn common smut with the highest degree of germination (D. S.) have more pathogenic power than isolates with lower degree of germination (D. G.), so producing new fungal biotypes are more possible in these isolates. In this study we examined the possibility of existence of various fungus biotypes of corn common smut in Lorestan province of Iran.

Material and Method

Planting selected corn lines in the field

Four selected corn lines, k17/2-3, k2817/1, k1259 and TVA926, were used for conducting the experiment (Table 1).

Table1. Characteristics of selected lines for study of corn common smut races in Lorestan province.

line	*origin*	*Ripeness period*
17/2-3 k	Karaj	premature
1/ 2817 k	Karaj	mature- premature
1259 k	Karaj	premature
926 TAV	Former Yugoslavia	too premature

The experiment was performed in Randomized Complete Block Design (RCBD) with 4 selected corn lines in an experimental field in Zarghan town (Shiraz, Iran). The field was prepared before experiment and Alachlor and Atrazine herbicides in the amount of 1.2 kg and 5 liter per hectare were used to control weeds. The number of planting lines of each line was two 7-meter lines with 75 cm spacing in each repetition, and there were 30 bushes in each line.

Preparation of inoculum:

Selection of pathogen isolates for the experiment

From among the isolates of each province, the isolates that had the highest germination percentages were selected to prepare inoculum (Warfied et al., 2006). In order to produce sporidia, 1 mg of each isolate was mixed with 0.323 mg/lit streptomycin sulfate and distilled water for 3 minutes and after disinfection was spread on the medium. Then, it was maintained for 24 hours in 30 ºc under dark conditions. In order to create artificial infection by wounding, after tasseling and pollinating, before the tassels dry, the bottom of each maize was cut by clippers by 2 cm, and 3 ml of suspension containing 106 sporidia was injected to the cut section (10 bushes of each line in each repetition) (Table 2).

Evaluating corn lines

After the grains have hardened, the inoculated maize in each line and repetition were separately harvested. Percentage of infection was determined by counting the number of diseased maize, and severity of infection was determined based on progress of disease in each maize through scaling method ranging between 0 (no infection) and 7 (100% infection). Statistical analysis was carried out after converting the data to arcsine square root of the data, and to compare the maens, Duncan's Multiple Range Test was used (Pope and McCarter, 1991; Pope and McCarter, 1992b; Banuett and Herskowitz, 1996).

Results and Discussion

In order to determine the existence of biotypes of *Ustilago maydis*, the isolates with the highest degree of germination than all other isolates of any province in Iran were selected.

Table 2. Characteristics of selected isolates of corn common smut pathogen for determination of races.

Number of isolates	*place of collection*	*Time of collection*	*sort*	*pathogen*	*severity of germination*	*percentage of germination after 8 days*

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1	Kohdasht/Abdovali	July 2008	704	*U. maydis*	3/97	79/4
17	Borojerd/Venay	September 2008	647	*U. maydis*	4/12	82/5
3	Khoramabad/Masour	September 2008	647	*U. maydis*	4/15	83
37	Norabad/Moradabad	September 2008	704	*U. maydis*	3/75	75
9	Poledokhtar/Champar	September 2008	704	*U. maydis*	4/13	82/7
43	Aleshtar/Kolahkaj	July 2008	678	*U. matdis*	3/9	78/2
8	Azna/Golgole	September 2007	678	*U. maydis*	4/1	82
22	Dorod/Jahanabad	July 2006	704	*U. maydis*	3/73	74/7
33	Aligodarz/Paresh	July 2006	704	*U. maydis*	3/6	72/3

Table 3: Variance Analysis of infection percentage and severity in corn common smut, *U. maydis*, in selected corn lines.

sources	factor A		factor B		Experimental error AB		
	mean squares	Degree of freedom	Mean squares	Degree of freedom	mean squares	Degree of freedom of mean squares	mean squares
Infection percentage	34229/53	3	3382/13	8	24	351/70	0/206
infection severity	147/435	3	3/947	8	24	0/443	0/002

Factor A: line
Factor B :isolate
Factor AB: interaction

According to the findings, isolate number 1 caused the highest and the lowest percentage of pathogenicity in k17/2-3 and TVA926 lines, respectively. In case of isolate 17, the highest degree was in k2817/1 line and the lowest degree was in k17/2-3 line. For isolate 3, the highest percentage was in TVA926 and the lowest degree was in k1263/1 line. In the cases of isolate 37, 9, 43, 8, 22 and 33 isolates, the highest infection percentages were in k1263/1, k17/2-3, k2817/1, k17/2-3 and TVA926 lines, respectively. Also, the lowest infection percentages were in k2817/1, k1259, TVA926, k1263/1 and k1259 lines, respectively. Zamani and Chogan (2000) studied the resistance of 60 inbred crosses of pure lines of selective compounds to common smut using injection in cob; and introduced K3165/2*Mo17 as sensitive composition and K1259/3*B73 as resistant composition. Renfro (1983) believes that, in terms of heredity, resistance to common corn smut is polygenic and he suggests that the method of crossing resistant lines with a sensitive line should be used in modification programs, and resistance evaluation in hybrids from F1 and F2 offspring be carried out. Meanwhile, there are so many haploid and diploid biotypes of fungus in nature that are created from hybridization and mutation in haploid and diploid stages; this vast amount results in ongoing assessment of sorghum selective genotypes in regions with high infection. Guly et al. (1980) reported that procedures of wounding the maize cause more infection than the procedures without wounding which go for spraying on the tassel, because the husk prevents the inoculation compound to reach corn seeds in spraying on tassels procedure, thus the infection decreases, so, these methods are not suitable for assessing the genetic resistance, because the low infection may be related to the escape mechanism not to natural resistance of corns. Similarly, Sulton et al. (2001) remarked that increase in disease percentage because of *Ustilago maydis* after various insects' attacks indirectly supports the efficiency of wounding methods in maize. Takhar et al. (2007), by comparing the infection percentage and severity percentage of *Ustilago*

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maydis in Yugoslavia, inferred that infection percentage is the variable criterion, since there were different results in two regions with respect to environmental conditions in terms of grouping. But it was not true about severity percentage. It demonstrates that severity percentage is a more stable trait in lines assessment while determining the reaction of various sorghum genotypes, because it specifies host's genetic resistance based on the involved active resistance. Depper et al. (1990) also believed that when the inoculation method is done through wounding the maize, first, the physiological resistance is assessed, and the next incidence in unwounded seeds depends on apparent resistance. Banuett et al. (1996), in assessing the resistance of 30 lines to common corn smut through two methods of wounding and injection (silk channel), concluded that various sorghum lines showed different reactions to the disease in terms of sensitivity and the disease severity in injection method in silk channel was lower than that of in wounding method. Thakur et al. (1989) believed that when the wounding method for inoculation is used, first, the physiological resistance is evaluated, and next incidence in unwounded seeds depends on apparent resistance; thus both apparent and physiological resistance are involved when selection of resistance to common corn smut after inoculation through wounding the maize is carried out. Therefore, the wounding method is the useful and recommendable method for studying heredity of resistance to disease and screening various genotypes. Delsorb (2005) introduced various biotypes of corn common smut in France. Hanfey et al. (2004) examined various biotypes of corn common smut in 10 regions of Cuba and identified 6 biotypes on 3 line3.

After physiological ripening of corn bushes and hardening of inoculated maize grains, each line was harvested separately and percentage and severity of infection of each treatment was determined. There were significant difference (p=%1) between isolates in terms of their ability to infect lines and the percentage and severity of infection. The table of variance analysis for percentage and severity of infection of isolates was drawn by SPSS software (Table3).

Isolates were divided into two separate groups, based on infection percentage, so as the first group included 6, 7, 9 and 8 isolates and the second group included 4, 5, 2, 1 and 3 isolates (isolate 33=1, isolate1=2, isolate17=3, isolate3=4, isolate 37=5, isolate 9=6, isolate 43=7, isolate 8=8 and isolate 22=9) (Figure 1).

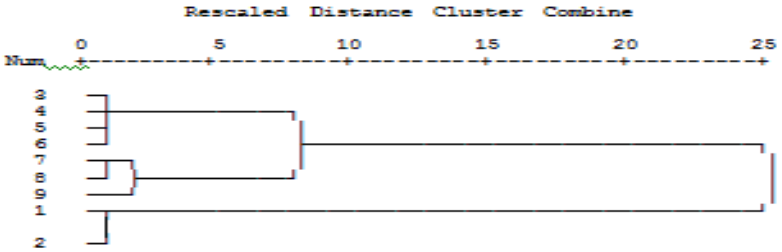

Figure1: Resulted dendrogram for isolates based on infection percentages characteristic.

Isolates were divided to two separated groups, based on disease severity, so as the first group included 3, 4, 5, 6, 7, 8 and 9 isolates and the second group included 1 and 2 isolates (Figure 2).

Based on percentage and infection severity, isolates 6, 7, 8 and 9 were placed is the same group.

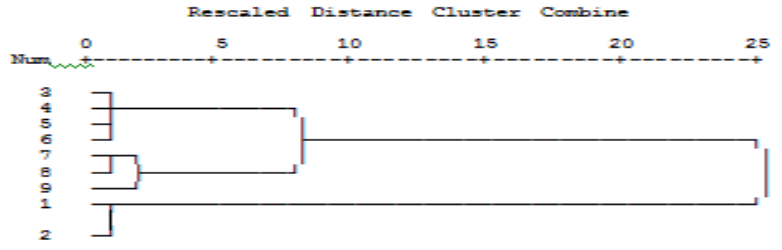

Figure 2: Resulted dendrogram for isolates based on infection severity trait

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Conclusion

According to the results, percentage and infection severity of various isolates in corn lines were different. The current evidence indicated that new biotypes of *U. maydis* exist in Lorestan province of Iran. We hope to identify the type of these biotype in the following studies.

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Multiple Shoot Induction by High Concentration of Sucrose in Stevia Micropropagation

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Abstract
Stevia rebaudiana Bertoni is a medicinal plants and commercially use as non-caloric and natural sweetener in drug and food industries. It is belongs to Asteraceae family and can be used as substitute of artificial sweeteners for diabetic patients. The effectiveness substance of this plant is called steviosid and can be used as natural sweetener instead of artificial sweeteners because of natural and non-caloric properties. However, at the first step low viability and low seed germination, limit their extensive cultivation. One of the effective methods to produce this plant is In vitro technology that solves the problem of large scale production of this plant. Sucrose is a very important component in in vitro culture media, serving as a source of carbon and energy. In this paper, effect of different concentrations of sucrose was investigated on micropropagation of stevia. For this purpose, MS culture medium with different concentration of sucrose (0.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 and 100 g/l) with two explants (Shoot tip and Node) were used. After 4 weeks shoot length, multiple shoot rate and number of root were evaluated. The analysis of collected data statically (ANOVA) showed that there is significant difference between studied parameters and different concentration of sucrose. Highest shoot length was obtained at 20-40 g/l sucrose (13.5 cm) from shoot tip explants. The maximum of multiple shooting rate was obtained in 60- 80 g/l sucrose (12 shoots per shoot cultured) from shoot tip explants. Also, highest number of root was observed at 70 g/l sucrose (12.3 roots per shoot cultured) from both explants.

***Keywords*:** Micropropagation, Multiple shoot, Stevia, Sucrose

Introduction

Medicinal plants are sources of important therapeutic aid for alleviating human ailments. With increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, interest in the use of plants and plant-based drugs has revived throughout the world. However, a large number of medicinal plants remain to be investigated for their possible pharmacological value. Most of the pharmaceutical industry is highly dependent on wild populations for the supply of raw materials for extraction of medicinally important compounds [13]. Due to a lack of proper cultivation practices, destruction of plant habitats, and the illegal and indiscriminate collection of plants from these habitats, many medicinal plants are severely threatened. Advanced biotechnological methods of culturing plant cells and tissues should provide new means of conserving and rapidly propagating valuable, rare, and endangered medicinal plants [10].

Stevia (*Stevia rebaudiana*) is a sweet herb and native plant of the Paraguay. It is a small perennial shrub that has been used for centuries as a bio-sweetener and for other medicinal uses such as to lower blood sugar. Stevia is a valuable medicinal plant species and it is being used for the treatment of diseases [6]. Its leaves have been traditionally used for hundreds of years in Paraguay and Brazil to sweeten local teas, medicines and as a 'sweet treat'. Japan is now the largest consumer of stevia leaves. in Japan stevia replaces the chemical sweeteners, aspartame etc, which were banned there in the 1970's. Other countries use lesser quantities of stevia [2]. Its leaves contain approximately 10% of steviosides which are intensely sweet compounds. Its white crystalline compound (stevioside) is the natural herbal sweetener with no calories and is over 100-300 times sweeter than table sugar. Stevia is an herb that is used extensively in various areas of the world as a non-caloric sugar substitute [5].

Stevia can be used wherever sugar is used, including in baking etc. They are ideal for diabetic and low calorie diets; in Japan 'diet Coke' uses steviosides. The plant has been successfully grown under a wide range of conditions from its native sub-tropics to Thailand and Indonesia and the cold northern latitudes of Leningrad and north China and Canada. In cold climates it is grown over the summer period as a transplanted annual (like tobacco), with a single harvest. In tropical areas it is a perennial (2 to 5 years) and multiple harvests per year are possible [14].

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Although stevia can be helpful to anyone, there are certain groups who are more likely to benefit from its remarkable sweetening potential. These include diabetic patients, those interested in decreasing caloric intake, and children. Stevia is a small perennial shrub that has been used for centuries as a bio-sweetener and for other medicinal uses such as to lower blood sugar. Currently, there is a high demand for raw material of this medicinal herb due to ever increasing diabetes disorder among the population. In order to meet the increased demand an efficient in vitro propagation of *Stevia rebaudiana* was established [9]. Plant tissue culture is an alternative method of propagation and is being used widely for the commercial propagation of a large number of plant species, including many medicinal plants [1].

The composition of culture medium is a major determinant of in vitro growth of plants. The mineral salts sucrose as carbon source and water are the main components for most plant tissue culture media. sucrose is a very important component in in vitro culture media and its addition is essential for In vitro growth and development of plants because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis [7]. Carbon sources are added to the culture medium because of the light energy deficiency and low CO2 concentration present in in vitro conditions. Sucrose concentrations of 20-30g/l are the most commonly used in tissue culture studies. The sucrose concentration chosen is dependent on the type and age of growth material; very young embryos require a relatively high sucrose concentration. Generally the growth and development increases with sucrose concentration, until an optimum is reached and then decreases at high concentrations. The most commonly used source of carbon is sucrose at a concentration of 2- 5%. Glucose and fructose are also known to support good growth of some tissues [3].

In vitro growth of plants is largely determined by the composition of the culture medium. Sucrose in culture medium has been considered the sole carbon source for the growth of cells, buds, shoots, and even plantlets. Sucrose enter the metabolism pathways and transformation of energy which are required for growth of cell. In plant tissue culture, sucrose serves as a carbohydrate supply to provide an optimal culture condition for cell. The main components of most plant tissue culture media are mineral salts and sucrose as carbon source and water [11]. Sucrose is a very important component in medium and its addition is essential for in vitro growth and development of plants because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis. The sucrose concentration chosen is very dependant on the type and age of growth material. Generally the growth and development increases with increased sucrose concentration, until an optimum is reached and then decreases at high concentrations. The most commonly used source of carbon is sucrose at a concentration of 2-5%. Glucose and fructose are also known to support good growth of some tissues [12]. The exogenous carbohydrates support growth of the nutrient medium serve as energy and carbon sources and these carbohydrates affect the physiology and differentiation of tissues. They also influence tissue growth, organ induction and differentiation. Impact of different carbohydrates with other constituents of nutrient media are reported in several studies. Among the sugars, sucrose is used as a principal carbon source for in vitro plant culture probably, because it is the most common carbohydrate in the phloem sap of many plants [4]. The objectives of this study were to investigate the influence of different concentration of sucrose in the medium on growth, multiple shoot and rooting of stevia plantlets.

Materials and methods

In this investigation were studied effective factors on micro propagation of stevia such as explants type and different concentrations of sucrose from achieving efficient protocol.

Plant material

Stevia rebaudiana Bertoni plants were procured from Agriculture Biotechnology Research Institute of Iran. In this experiment, shoot tip and node segments were used as explants.

Explants sterilization

The shoot tip and node explants were washed in tap water and gently rinsed with 20% (v/v) extra and surface sterilized in 0.1% sodium hypochlorite solution for 10 min and then rinsed with five changes of sterile distilled water.

Culture establishment

In order to different concentrations of sucrose with two explants were used. The culture medium consisted of MS [8] salts, vitamins, 3% (w/v) sucrose and the pH of the media was adjusted to 5.6 with 0.1 N NaOH or HCl before adding of agar and autoclaved at 121◦C for 15 min. After surface sterilized shoot tip and node explants were cultured on MS medium completed with different concentrations of sucrose (0.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 800.0, 90.0 and 100.0 g/l) for proliferation. The cultures were incubated at 24±2 ◦C under 16/8 h (light/dark cycle) photoperiod (60 µE m−2 s−1) and irradiance provided by cool-white fluorescent tubes.

Hardening and acclimatization

Well rooted plantlets were carefully removed from culture vessels, washed under running tap water to remove the remnants of agar. After proper removal of agar dip the plantlet in the sterile water for 1 minute and transferred to tray containing sand. For 1 week, the potted plantlets were kept under transparent polythene membrane to ensure high

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humidity, and then they were kept in open in diffuse light for hardening. After 7 days, the surviving plants were transferred to pots containing garden soil and maintained in green house for acclimatization.

Statistical analysis

Experiments were done in factorial based on completely randomized design (CRD) with 3 replications and observations were recorded after the 4 weeks. The analysis of variance (ANOVA) was performed using SAS program. The differences among means were determined by Dunkan Test at 1% significant level.

Results and Discussion

Different concentrations of sucrose were used to study their effect on micro propagation from explants type. A perusal of statistical analysis of variation reveals that effect of different concentration of sucrose on growth plant of stevia (Table 1).

Table 1: Analysis of variance (ANOVA) for micro propagation parameters of *Stevia rebaudiana*

Source of variance	Degrees of freedom	Mean square		
		Shoot length	Number of root	Multiple shoot rate
Sucrose	10	56/08**	99/44**	84/89**
Explant	1	231/84**	25/47**	72/13**
Sucrose × Explant	10	3/52**	1/23**	15/47**
Error	44	0/31	0/27	0/34
٪CV		5/8	11/6	17/1

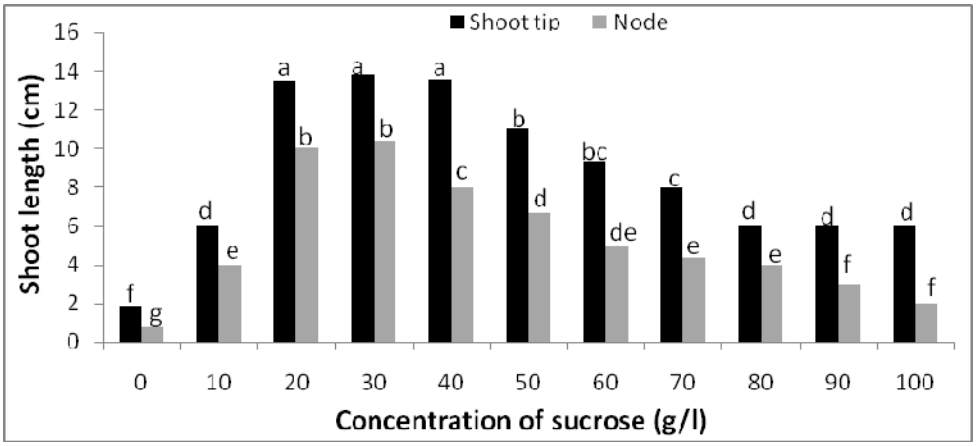

Fig. 1. Effect of different concentration of sucrose on the shoot length

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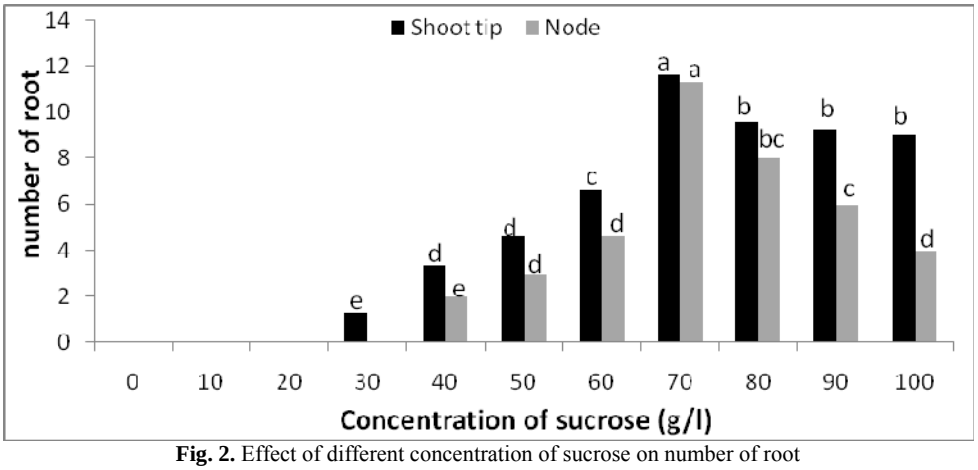

Fig. 2. Effect of different concentration of sucrose on number of root

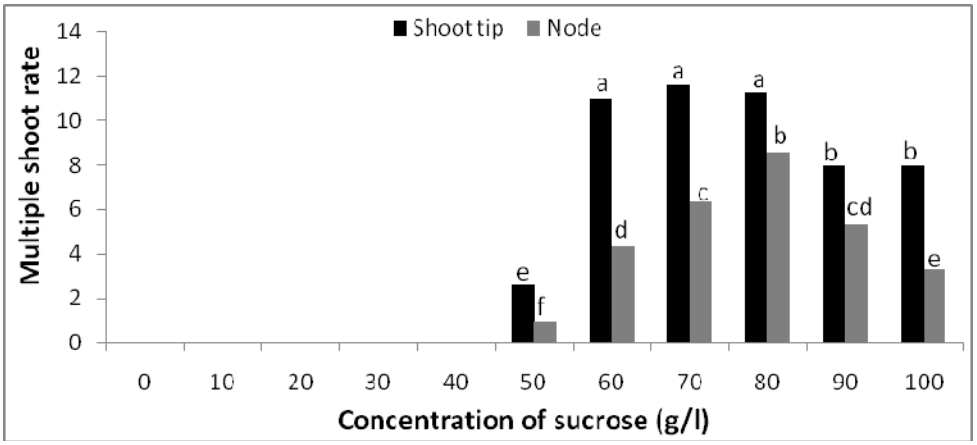

Fig. 3. Effect of different concentration of sucrose on multiple shooting

Shoot length

Interactive source variation of sucrose × explants showed significance at $p<0.01$ for shoot length of in vitro produced plantlets. Observations showed that low concentrations of sucrose enhance the shoot length. Highest shoot length were obtained at 20.0, 30.0 and 40.0 g/l sucrose from shoot tip explants (Figure 1). Shoot length were decreases at higher concentration of sucrose. May be due to the inhibition of organogenesis

Number of root

Effect of different concentrations of sucrose was investigated for induction of root. Observations showed that the high concentrations of sucrose cause stimulation increases rooting of the plant stevia. Maximum number of roots per explant was observed when the explants (Shoot tip and Node) were cultured on MS medium supplemented with 70.0 g/l sucrose (Figure 2).

Multiple shoot rates

The effect of different concentration of sucrose on multiple shoot rate process of explants is more similar to variations observed in number of root and among all the treatments. The maximum of multiple shooting rate was obtained in 60.0, 70.0 and 80.0 g/l sucrose from shoot tip explants. A more than the tenfold increase in the multiple shooting rates per explants (12 shoots per shoot cultured) was observed when the explants were cultured on MS medium supplemented with high concentration of sucrose (Figure 3).

Conclusion

The growth and multiplication of shoots in vitro are affected by many factors, one of which is the concentration and type of exogenous carbon source added to the medium. The

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carbon sources serves as energy and osmotic agents to support the growth of plant tissues. In addition growth and root initiation are highly energy requiring processes that can occur at the expense of available metabolic substrates, which are mainly carbohydrates. Sucrose has been proved to be better for shoot proliferation than other carbon sources in micro propagation of several plant species. Finally, there is a positive relationship between increase in sucrose concentration and increase in number of rooting and multiple shooting rates was verified, This increase in the amount of sucrose in the culture should be taken with caution and should not be progressive, because, high sucrose concentrations in in vitro cultures favor carbohydrate accumulation and hinder photosynthesis. Sucrose concentration influenced growth and accumulation of biomass of stevia plantlets propagated in vitro. So that in this present study high shoot length was obtained in MS medium supplemented with low concentration of sucrose. Also Shoot length were decreases at high concentration of sucrose, may be due to the inhibition of organogenesis. However, further research is needed to know the impact of carbon sources on development of shoots and the physiological changes during growth of stevia.

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Airborne Fungi and their Effect on Plant Pathogen

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Abstract

In order to identify the airborne fungi in the state of Lorestan, Islamic Republic of Iran, circular trays were used for sampling purposes in five different cities (namely Kouhdasht, Khorramabad, Azna, Boroujerd, and Poldokhtar) during sand-dust storm a number of eight times with one-month intervals. Soil suspension was then isolated from the samples and cultured in a PDA containing 323mg/L Streptomycin sulfate to isolate fungi. The analysis of the data identified fungi of the following type: *Alternaria alternate- Penicilium italicum- Aspergillus niger- Clostridium cladosporides- Fusarium avenacum -Fusarium graminearum - Drichlera biseptata - Aspergilus fumigates- Sclerotinia cepivurum - Curvularia lunata– Penicilium digitanum- Sclerotinia sclerotiorum Rizoctonia solani- Bipolaris sorokiniana- Sclerotinia sclerotiorum- Rhizopus stolonifer- Mucor rouxii- Drechlera teres- Curvularia ovoides – Trichoderma virens Trichoderma harzianum* with - *Fusarium avenacum* and- *Rhizopus stolonifer* being the most frequently observed and *Alternaria alternate* and *Drechlera teres* the least. In addition, the analysis revealed the highest mean for fungi concentration to have occurred in August.

Introduction

Researches show the source of sand-dust storms to be dry and semi-land especially Middle Eastern Sahara and Mongolia. Other sources have also been reported to be northwestern America and Australia (Hong, 1993). Frequency analyses of sand-dust storm weather in Iran assign the highest frequency to the central pits (Annon, 2009a). The main responsible sources for sand-dust storms in western parts of Iran such as the states of Lorestan, Ilam, Khouzestan, and Boushehr are the close-by deserts including the Syrian Sahara, Iraq, and northern Saudi Arabia (Annon, 2009b). Dust has been identified as the main carrier of toxic fungi (O'Hara et al., 2000). Sand-dust storms have also major negative influences on agriculture (Engelstaedter and Tegan, 2006; Krueger et al., 2004; Wang et al., 2005). Atmospheric dust decreases exposure to sunlight by a degree of %5-30 (Ye et al., 2003). During sand-dust storms, nutritional and organic substances in the soil perish which leads to lower yield (Xuan et al., 2004). Moreover, the damage to wheat and corn crops in Iran due to airborne fungi is estimated to be %15-20 (Annon, 2009b). According to a research on two cases of dust-storm weather, the dominant types of fungi were *Aspergillusniger, A. fumigatus, A. tami, Alternaria alternate, Penicillium* spp and *Coleterodium* spp, mostly well-known fungi (Schlesinger et al., 2006). A study on the airborne microorganisms in Virginia Islands revealed that, of the isolated microorganisms, %25 were plant pathogens and %10 opportunistic human pathogens (Griffin et al, 2001). Basidiomycetes fungi frequent dust storms more than does any other type of fungi, followed by Ascomycetes which add up to %30-40 airborne fungi (Griffin et al., 2005). The Fusarium spp have been found responsible for leaf spots, windburn, and root rot, the RIZOPOS for fruit and post-harvest soft rot, and the Rhizoctonia spp for canker rot, stolons, and rhizomes (Elahinia, 1993). In light of these severe consequences of airborne fungi with regard to plant pathogen, the present study attempted to isolate and identify recent dust-storm related fungi in different regions of the State of Lorestan, to identify the pathogenic roles of which requires further studi

Materials and Methods

Sampling and Strain Isolation: For sampling purposes followed by isolation and identification processes, one region from each city was randomly selected where four circular trays were then put, each a certain distance away from the others. The sampling was carried out during eight months with one-month intervals. To isolate the fungi from the samples serial dilution was employed (sterile distilled water was used as control instead of suspension). Every 1gr of soil was diluted in 9mLit of sterile distilled water, a process reiterated a number of 6 times. Once this suspension was prepared, 1mLit of the

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surface suspension was diluted in 9mLit of sterile distilled water, itself to be diluted in 9mLit of sterile distilled water. The serial dilution was done enough times to reach a concentration of 10^{-6}. Sterile pipets were use throughout the process. Afterwards, 1mLit of the suspension was gradually cultured in a PDA containing 0.03mg/L Streptomycin sulfate to isolate fungi, which was stored in an incubator at 25 C to be later isolated and sub-cultured again.

Purification and Isolation of Strains: The fungi were purified using the single-spore method in a water agar environment and were eventually identified by key fungi identifiers (Nelson et al., 1883; Simons, 2007; Barnet and Hanter, 1995; Schlesinger et al., 2006; Leslie and Summerell, 2006). 12 different species of fungi were isolated and identified in the samples.

Analysis and Data: The completely randomized design was the approach taken to the tests, run for a number of 5 times. The soil suspension was isolated from the soil from the trays (with sterile distilled water as control instead of suspension). The analysis of the data was done by Microsoft Excel 2010.

Results and Discussion

The present study isolated and identified from dust-storm samples the following types of fungi: *Alternaria alternate- Penicilium italicum- Aspergillus niger- Clostridium cladosporides- Fusarium avenacum -Fusarium graminearum - Drichlera biseptata - Aspergilus fumigates- Sclerotinia cepivurum - Curvularia lunata– Penicilium digitanum- Sclerotinia sclerotiorum Rizoctonia solani- Bipolaris sorokiniana- Sclerotinia sclerotiorum- Rhizopus stolonifer- Mucor rouxii- Drechlera teres- Curvularia ovoides – Trichoderma virens Trichoderma harzianum* (Fig. 1 and 2). The percentile values for the presence of the fungi differed with time. The predominately observed types at all times were: : *Alternaria alternate- Penicilium italicum- Aspergillus niger- Clostridium cladosporides- Fusarium avenacum - -Drichlera biseptata - Sclerotinia cepivurum - Curvularia lunata - Sclerotinia sclerotiorum Rizoctonia solani- Bipolaris sorokiniana -Rhizopus stolonifer- Mucor rouxii- Drechlera teres- Curvularia ovoides – Trichoderma*; nonetheless, other types were sporadically identified at different times: *Fusarium graminearum* was identified in samples taken in the months of Bahman, Esfand, and Ordibehesht in the city of Kouhdasht; in Esfand and Khordad in the city of Khorramabad; and, in Farvardin in Poldokhtar. The *Trichoderma harzianum* species, on the other hand, was seen in Farvardin in Khorramabad, the *Drichlera biseptata* in Ordibehesht in Poldokhtar and in Esfand in Nourabad, the *Sclerotinia cepivurum* in Khordad and Shahrivar in Nourabad, the *Penicilium digitanum* in Khordad an Azna, and *Curvularia lunata* in Shahrivar in Nourabad, in Khordad in Poldokhtar, and in Tir in Khorram abad. It is worth noting that the *Alternaria alternate*, *Aspergillus niger*, and *Fusarium avenacum* were observed at all times, and that the *Fusarium avenacum* and- *Rhizopus stolonifer* were the most frequently observed with *Alternaria alternate* and *Drechlera teres* the least (Diagram 1). Studies show that the *Drichlera biseptata* and *Curvularia lunata* were not common to the studies areas.

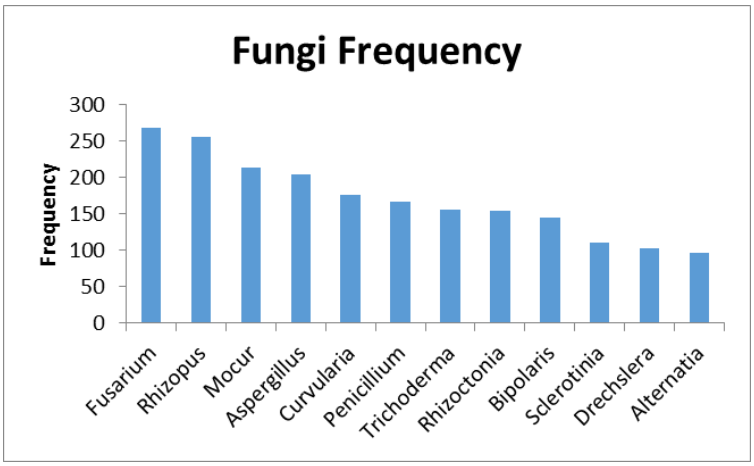

Diagram 1. Percentile frequency of airborne fungi in cities of State of Lorestan

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The results presented are from the analysis of variance of data (Table 1).

F	Square Means	Square Sums	df	Source of Variation
4.49*	3496.1175	13984.47	4	Reiteration
4.89**	3809.455	15237.82	4	Factor A (Cities)
-	778.8925	12462.28	16	a error
-	-	38403.57	24	Main plots
0.812^{ns}	38.3308	268.315	7	Factor B (Time of Year)
0.147^{ns}	6.945	194.46	28	AB Interaction
3.832**	180.8861	5064.81	28	RB Interaction
-	47.2146	5288.04	112	b error
-	-	10815.625	175	Borderline plots

Table 1. Results from analysis of variance

The results reveal an interaction effect between each city's dust-storm pollution in different months and the corresponding fungi pollution, meaning that a quantitative increase in dust storms results in increased fungi population. Basically, the mean fungi presence for Mordad exceeds those of other months; in addition, the least-polluted city with respect to fungi presence was Kouhdasht followed closely by Azna, which is arguably due to the relatively lower dust storm rate of the city (Diagram 2).

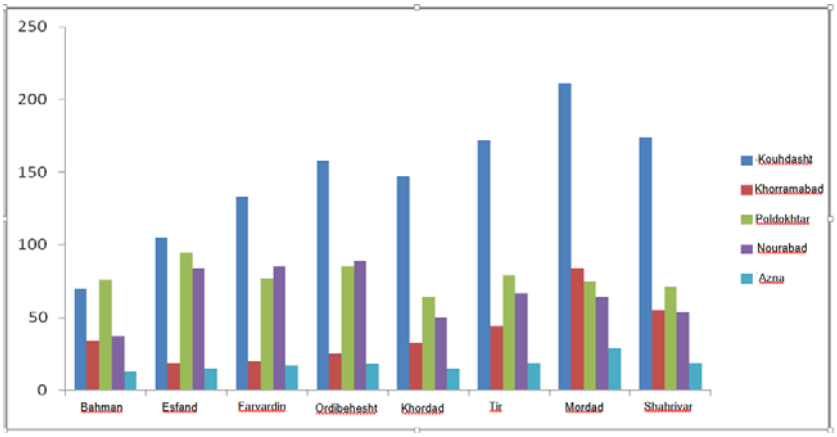

Diagram 2. Airborne fungi pollution for thr cities in the State of Lorestan

Data shows the most polluted city and the most polluted months to be respectively Kouhdasht and Mordad. Of the cities covered in this study, Kouhdasht was closest in distance to Iraqi borders which feature very high dust-storm weather (Diagram 3).

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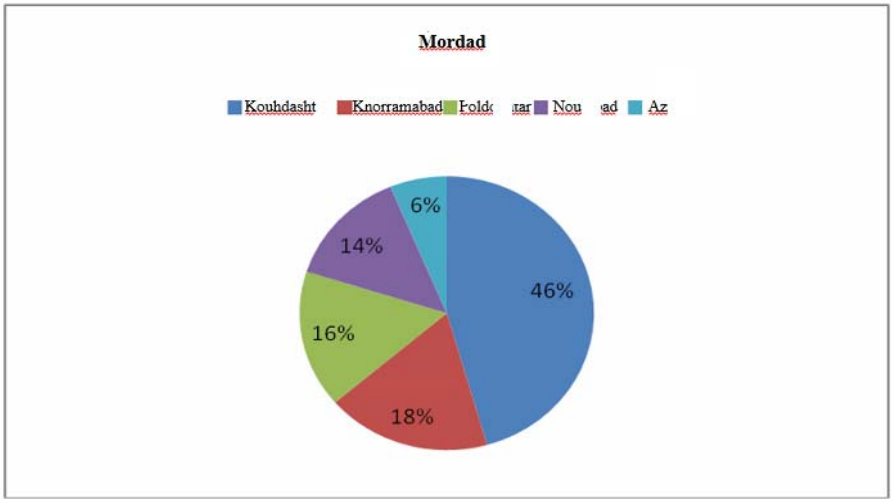

Diagram 3. The airborne-fungi pollution of the cities of the State of Lorestan

This suggests that the interactions between the air, plants, and microbial pathogens can affect plants in many pathological capacities (Manning, 2009). A study in Taiwan identified spore fungi of the types *Penicillium*, *Aspergillus*, *Nigrospora*, *Arthrinium*, *Culvularia*, *Stemphylium*, *Cercospora* and *Pithomyces* in dust storms. Moreover, it was shown that an increase in dust storm concentration led to higher quantities of spore fungi such as *Penicillium* and *Aspergillus* (Pei et al., 2004). Dust storm increases the occurrence of leaf spot and darkens grapes leaves (Manning, 2006). During dust storm weather the nutritional and organic substances in the soil vanish which leads to significantly lowered yield (Bayat and Jamali, 2011).

The study carried out over Virginia Islands in 2009 on dust-storm microorganisms showed %25 of the microorganisms to be plant pathogens (Griffin and Garrison, 2009). Biological measures are the key to neutralizing dust storm effects. As the results here suggest, the *Alternaria*, *Fusarium*, and *Aspergillus*, highly potent pathogenic, allergic, and toxic agents, were present regardless of the time of the year. 13 different types of plant pathogenic fungi were detected in the areas covered by the study within the time period when the study was done. According to current resources, *Drichlera biseptata* and *Curvularia lunata* were not mong the fungi mycobiota of the area; it seems safe to conclude that their persistent appearance has to do with dust storms, resulting in a critical situation with regard to local (decreased) crop quality and quantity (Azadi, 2013).

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