International Journal of Agriculture Innovations and Research
Volume 2, Issue 2, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 28/08/2013 | Accepted on : 12/09/2013 | Published : 17/09/2013

# Control Effect of Rare Earth Elements on Crown Rot Disease of *Aspergillus Niger* in Groundnut Plants (*Arachis Hypogaea* L.)

**E. S. Challaraj Emmanuel**
Department of Microbiology,
St. George College, Banaswadi, Bangalore-560043, India
Email: emmyesc@yahoo.com

**S. Maruthamuthu**
Corrosion Protection Division,
Central Electro Chemical Research Institute,
Karaikudi-630006, India

***Abstract*** **– Five soil-transmitted pathogenic fungi (*Aspergillus niger*) were isolated and selected to investigate the effect of Rare earth elements on their disease causing ability. The present study reveals the response of leguminous plant (*Arachis hypogaea* L.) to monazite soil containing Rare earth elements. A significant increase in plant biomass, total chlorophyll content and stress resistant product (phenols) was observed. The results indicated that seeds soaked with REEs show significant disease controlling effects on root rot by the pathogenic fungi. The activities of two pathogenic enzymes (cellulase and pectinase) of the fungi were reduced by the application of REEs. The results indicated that REEs in agriculture not only promote the biomass but also control the soil pathogens.**

***Keywords*** **– Cellulase, Crown Rot, Pectinase, Rare Earth Elements.**

## I. INTRODUCTION

Control of root diseases is generally based on the application of fungicides (Keureni et al., 1992), use of resistant cultivar (Dong and Cohen, 2002) and use of some agronomic techniques (Riegel and Note, 2000). With the increasing difficulties in breeding novel resistant cultivars (Reuveni and Keuveni, 1995), reducing pesticide levels in agricultural products and the environment, together indicate the need for alternative methods of disease control. One of the potential methods to reduce the fungal diseases is the application of rare earth.

The infection process of the pathogen involves the interaction between the pathogen and the host plant. Under suitable environmental conditions, the ability of a pathogen to infect a host plant depends on the amount of pathogenic factors and their activities. The main pathogenic factors of the pathogen have been known as enzymes, toxins, and hormones. Pectin, cellulose, semi-cellulose, and wall protein are the major components of the cell wall, which form the barriers for pathogen invasion. Pectinase, proteinase, and cellulase are important cell-wall degradation enzymes and thereby act as pathogenic enzymes. Their activities are related to the pathogenic ability of the pathogenic fungi to invade plants. Some pathogens enter into the host plant passively through natural holes or wounds.

Rare earth (RE), a kind of natural mineral has been used as a beneficial element to crops RE has been found to have some controlling effects on many plant diseases. Chinese researchers reported that RE have a controlling effect on the diseases of rice blight and cabbage soft decay in 1980. Lanthanum had significant direct inhibition on pathogenic fungi, the inhibitory activity or toxicity of Lanthanum is even found to be approximately equal to some common organic fungicides (Mu et al, 2006). The proper concentration of rare earth could promote growth and development of plant, increase the physiological activity and enhance the resistance to bad environment (Bai and Deng, 1995). The treatment of seed soaking with REE solution increased the resistance to seed rot, bud rot, and root rot. Besides the reduction and alleviation effects of disease, the reason of promoting plant growth and development might also be the increase in chlorophyll content of leaves and in photosynthesis, as well as the improvement in root growth, root activity and nutritive absorption.Applying appropriate amount of rare earth elements could not only promote seed germination and root development, increase plant biomass, but also improve harvest quality and plant resistance against stress. Rare earth elements also enhance chlorophyll content, thereby improve photosynthetic rate (He and Xue, 2005).

*Aspergillus niger* is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans. This organism is a soil saprobe with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose. Rot diseases are mostly fungal diseases which are soil and seed borne. It causes disintegration of fruit and seed tissues. *Aspergillus niger* causes pre emergence rotting of groundnut seeds sudden wilting followed by shredding of the collar region in young seedlings. Infected collar regions are profusely covered with black masses of mycelium and conidia (Gibson, 1953).

The major objective of the present study is to determine the efficacy of REEs on the phytopathogenic activity of *Aspergillus niger*. Bio-control of crown rot disease in *Archis hypogaea* using REEs was evaluated with enzymatic activities such as pectinase and cellulase. Further the studies were concentrated on the impact of REE on plant biomass, chlorophyll content and accumulation.

## II. MATERIALS AND METHODS

### *A. Assessment of REEs in the soil*

REEs rich soil samples were collected from Manavalakurichi (Kanyakumari District,Tamil Nadu, South India). The samples were collected in sterile

polythene cover and brought to the laboratory for analysis. The known weight of collected soil samples was acid digested with 3:1 ratio of HCl and $HNO_3$ respectively, and volume was made up to 10 ml with distilled water (Hill et al., 2002). The digested soil samples were analyzed for the evaluation of REEs concentration using Inductively Coupled Plasma-Mass Spectrometry (Perkin Elmer Sciex ELAN DRC II.ICP-MS).

*B. Isolation and enumeration of Aspergillus niger.*

Five locations were selected for survey of *Aspergillus niger* prevalence in Madurai district, Tamil Nadu, India. The selection was based either on the cultivation of groundnut or groundnut happening to be a component in the cropping system of that locations. The soil samples (0.5 kg soil) were collected after the harvest and *Aspergillus niger* population was isolated and enumerated by serial dilution method using Czapek-Dox-Agar. The plates were incubated at 28 °C and fungi count was estimated after 5 days and again after 8 days to count the slow growing fungal species. The isolated fungi were identified according to their morphological characters (Barnet, 1960).

*C. Assessment of biomass and REEs fractionation*

The seeds of *Arachis hypogaea* were treated with 1% sodium hypochlorite and rinsed clean of any traces of sodium hypochlorite. They were soaked in soil solution (100 mg $L^{-1}$ ) rich in REE for 24 hours. The treated seeds were placed in sterile petri plates containing cotton soaked with sterile water to provide moist condition for sprouting. Then they were allowed to grow on pots containing garden soil (sterilized) till the growth achieved with complete root and shoot formation. The physical parameters are the direct measurements of the plant biomass (with and without treatment of REEs). The overall biomass of groundnut plant was evaluated using parameters like length of root and shoot, fresh weight and dry weight of plant. Studies of various growth parameters, such as, Shoot length (Arts and Marks, 1971), Root length, fresh weight, dry weight, number of nodules, etc., were done at 90 days interval. The presence of REEs was measured in different parts of plant i.e., leaf, root and shoot. Fractionation of plants was done after acid digestion with 3:1 ratio of HCl and $HNO_3$ (Wang *et al.,* 2001), and measured using Inductively Coupled Plasma Optical Emission Spectrometry (Optima 5300 DV ICP- OES).

*D. Estimation of chlorophyll in leaves*

Chlorophyll was extracted with 80 % acetone and the absorption at 663 nm and 645 nm were read in spectrophotometer. Using the absorption co-efficient the amount of chlorophyll was calculated. One gram of leaf was extracted with 20 ml of 80 % acetone and centrifuged at 5000 rpm for 5 minutes. The supernatant was transferred to a fresh flask until the residue was colourless. The supernatant was made up to 100 ml with 80 % acetone. The absorbance of the solution was read at 645 nm and 663 nm against 80 % acetone blank and the amount of chlorophyll was estimated (Witham et al., 1971).

*E. Estimation of phenols in leaves*

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium to produce blue coloured complex quantified at 650 nm. 0.5 gm of leaves were homogenized in 10X volume of 80 % ethanol, then centrifuged at 10000 rpm for 20 minutes. The residue was reextracted with 80 % ethanol; the supernatant was evaporated to dryness and dissolved in distilled water with known volume (0.5 ml) of Folin-Ciocalteau reagent. After 3 minutes 2 ml of 20 % sodium carbonate was added and the mixture was placed on a boiling waterbath for 1 minute. The amount of phenol in the extract was then quantified using a spectrophotometer at 650 nm. A standard catechol solution corresponding to 2- 10 □g concentration was added with Folin-Ciocalteau reagent and sodium carbonate. A standard curve was constructed using an electronic calculator on the linear regression mode using which the concentrations of phenol in samples were read. The values were expressed as milligrams of phenol per gram of leaf (Mallik and Singh, 1980).

*F. Evaluation of pathogenicity of Aspergillus niger on groundnut*

The sprouted seeds (both treated with REE and untreated) of groundnut plant were inoculated with *A.niger* culture at the apex portion, and incubated for 2 hrs. After incubation the infection was observed by the colour change (black colouration). The Microscopic observation of the mycelium (infection thread) was analysed by making a thin smear stained with Lactophenol cotton blue. The pathogenecity of the *Aspergillus* sp. isolated from five different locations were evaluated by their ability to cause rot in groundnut seedlings and the production of enzymes such as Pectinase and cellulase associated with plant pathogen. The enzymatic activity was evaluated both in the presence and absence of REE (monazite soil rich in LREE – 10 mg $g^{-1}$).The percentage of fungal infection in each plant was determined by gridline intersect method( Giovannetti and Mossae,1980), using the formula.

Percentage of Infection = Total number of infected roots intersecting gridline/Total number of roots intersecting gridlines X 100

*G. Enzymatic studies to evaluate the pathogenecity of isolated strains of Aspergillus niger*

Czapek's medium devoid of sucrose with 3% pectin for the detection pectinolytic enzyme or 1% cellulose powder for the detection of cellulolytic enzyme was prepared. Czapek's medium with sucrose alone served as control. The media were dispensed in 50 ml aliquots in 250 ml Erlenmeyer flasks and inoculated with 8mm culture disc of the *Aspergillus niger* isolated from five different locations. For each treatment three replicates were maintained. After 7 days of incubation at room temperature (28 ±2° C) the mycelium was removed and the culture filtrates were retained for enzyme study.

*Pectinase Activity*

The Pectinase activity of the culture filtrate was determined by changes in viscosity of citrus pectin. To 4 ml of freshly prepared 1% citrus pectin dissolved in boric acid buffer at pH 8.6, 1 ml of Tris acetate buffer and 2ml of the culture filtrate were added. The mixture was

transferred to an Oswald-Fenste Viscometer and loss in viscosity was determined after 2 hours of incubation.

*Cellulase Activity*

The cellulase activity of the culture filtrate was estimated using Carboxy Methyl Cellulose (CMC). To 4 ml of CMC solution(0.5%), 1ml of Acetate buffer a (pH 4.8) and 2 ml of the culture filtrate were added and transferred to an Oswald-Fenste Viscometer placed in a water bath at 30±1° C. The reduction in the viscosity of CMC was determined.

## III. Results

The processed monazite soil samples recovered from south coastal regions of India (Kanyakumari District) were analyzed for the presence of REEs by ICP-MS analysis. Among the 12 lanthanides analyzed Lanthanum, Cerium and Neodymium, were present in higher proportions in the soil sample (144.16, 285.21 and 119.50 ppm respectively) (Table 1).

The physical parameters of the plant biomass were evaluated (root and shoot length, fresh and dry weight of root and shoot) with and without treatment of REEs. The results show a significant increase in root and shoot length, fresh and dry weight of the REE treated plants. It also reveals a prominent increase in number of root nodules and their fresh and dry weight when treated with REEs, compared to the untreated seeds (Table 2).

Table 1: ICP-MS for soil sample

| S. No. | Analyte | Mass | Conc. Mean (ppm)* |
|---|---|---|---|
| 1. | Pr | 141 | 33.92 |
| 2. | Yb | 172 | 1.87 |
| 3. | Eu | 151 | 0.51 |
| 4. | La | 140 | 144.16 |
| 5. | Ce | 146 | 285.21 |
| 6. | Sm | 147 | 19.03 |
| 7. | Gd | 157 | 11.81 |
| 8. | Tb | 159 | 1.03 |
| 9. | Dy | 163 | 3.30 |
| 10. | Er | 166 | 1.60 |
| 11. | Tm | 169 | 0.31 |
| 12. | Nd | 165 | 119.50 |

*Results obtained as such from ICP MS

Table 2: Assessment of plant biomass (*Arachis hypogaea*)

| Treatments | Shoot length (cm) | Root length (cm) | Plant fresh weight (g) | Plant dry weight (g) | Nodules Plant $^{-1}$ | Weight of Nodules (mg) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Fresh | Dry |
| With REE | 24.9 ± 2.12 | 12.23±1.02 | 10.0± 0.85 | 0.84± 0.02 | 17.6± 0.12 | 0.109± 0.005 | 0.032± 0.004 |
| Without REE | 18.5±1.4 | 7.8± 0.95 | 6.84± 0.52 | 0.61± 0.01 | 6.4± 0.10 | 0.054± 0.004 | 0.016± 0.001 |

Values are average of three observations ± SD
Statistically significant (P<0.05) compared to untreated seeds

Based on earlier ICP-MS analysis, three elements were considered for further bacterial accumulation studies viz., Lanthanum, Cerium and Neodymium. The accumulation in the different parts of plants analyzed by ICP-OES showed increased levels of Lanthanum and Cerium (7.08 and 6.95 ppm) in roots than in shoot and leaf of *Arachis hypogaea*. Whereas, the concentration of the other element namely Neodymium was below the detectable limit (Table 3).

*Aspergillus niger* was isolated from five different locations of Madurai District (Tamil Nadu,India) and were confirmed by microscopic observations (Figure 1). The number of colonies formed on Czapek-Dox-Agar plates was enumerated. The percentage of infection by the isolates were also carried out by intersect gridline method (Table 4).The production of stress resistant end product (phenol) was measured using the method adopted and expressed as phenol equivalents in mg/g.

Table 3: Accumulation of REEs in plant tissues Values are average of three observations ± SD

| S. No. | Sample | REEs accumulation (ppm) | | |
|---|---|---|---|---|
| | | La | Ce | Nd |
| 1 | root | 7.08±0.03 | 6.95±0.01 | 1.92±0.002 |
| 2 | shoot | 5.01±0.01 | 4.82±0.02 | 1.21±0.001 |
| 3 | leaf | 1.92±0.01 | 0.96±0.01 | 0.83±0.001 |

Table 4: Enumeration of soil borne *Aspergillus niger* isolates at five different locations

| Location | Total number of fungal colonies at $10^{-3}$ dilution (Number of colonies/ plate) | Total number of *Aspergillus sp* colonies at $10^{-3}$ dilution (Number of colonies/ plate) | Total number of *Aspergillus niger* colonies at $10^{-3}$ dilution (Number of colonies/ plate) |
|---|---|---|---|
| M1 | 41.2±1.22 | 25.6±1.01 | 2.6±0.01 |
| M2 | 38.9±1.06 | 21.4±0.92 | 5.6±0.05 |
| M3 | 25.2±1.01 | 13.8±0.87 | 2.7±0.01 |
| M4 | 42.4±1.31 | 16.6±0.65 | 1.8±0.01 |
| M5 | 27.6±1.05 | 9.7±0.42 | 0.92±0.001 |

Values are average of three observations ± SD

The counts for fungi should be multiplied by $10^4$ and represent the numbers of organisms per gram of oven-dried soil

The total content of soluble phenols was estimated and was found to higher in plants treated with REEs compared to the other trials. Seed treated with REE alone show two fold increases in the production of phenol compared to others. It was found that an increased level of chlorophyll content in leaves of REE treated plants than the untreated (Table 5).There were significant disease controlling effects such as reduction in pectinase and cellulose activity of the fungal isolates when seeds were soaked with REE solution.

Table 5: Estimation of total chlorophyll and phenol content in leaves of *Arachis hypogaea*

| Parameters | With Element | Without Element |
|---|---|---|
| Total Chlorophyll (mg/gm) | 1.85 ± 0.025 | 1.12 ± 0.006 |
| Total Phenols (mg/gm) | 12.02 ± 0.110 | 10.10 ± 0.24 |

Values are mean ± SD of triplicates.
Statistically significant (P<0.05) compared to untreated seeds

Thus relating to the controlling effect of root rot disease in plants by REE. Soaking the seeds in the REE rich solution increase the emergence rate of seedlings and improve the growth of *Arachis hypogaea.* There was remarkable control effect of REE on *Aspergillus niger*. Plants treated with REE decrease the infection rate of the fungus remarkably (Table 6). The formation of black coloured mycelial masses at the infected collar regions of *Arachis hypogaea* indicates the infection of *A.niger.* This was confirmed by microscopic observations of the infection thread of the fungal pathogen (Figure 2).

The total pectinase activity decreases on account of the strong inhibition of Lanthanum, Cerium and Neodymium to mycelia growth and enzyme exudation. The cellulase activity of *Aspergillus niger* was inhibited with relation to Lanthanum concentration in the soil. The absorption of REE by seeds not only inhibits mycelia growth, also decreases the pathogenic ability of the fungi by reducing their enzymatic activities.

## IV. DISCUSSION

The reports of Parthasarathy et al. (1986) state that the lanthanide contents of monazite soil (Southern coastal regions of India, Kanyakumari District) contains significant amount of composition in terms of REEs. The REE distribution of monazite shows the general enrichment of LREEs which is due to preferential incorporation of LREEs relative to the HREEs in monazites. In the present study, a similar type of distribution of LREEs especially La, Ce and Nd were found to be rich in the monozite soil samples. Jeya et al. in 2008, reported the presence of Ce and Nd in monazite sample which was comparatively higher than other REEs in the soil samples of South coastal regions of India. Hence in the present study La, Ce and Nd were considered for their accumulation and impact on growth of plants of *Arachis hypogaea*. Soaking of groundnut seeds in REEs solutions of low concentrations show moderate effect and the best effect in growth of plants was observed in seeds soaked in high concentrations. Jarvan in 2002 observed that the soaking of seeds in REEs solutions at different time intervals.

Increased rare earth concentration in root, leaf and stem following the application of rare earth fertilizers were also observed by (Wen et al., 2001). No obvious accumulation of individual rare earth elements was induced at concentrations below 10 mg/kg soil, whereas doses of 50 mg/kg soil resulted in a preferred accumulation of lighter rare earths in both roots and tops of corn (Xu et al., 2003). Compared to cerium, lanthanum was shown to be taken up selectively by the plants. In addition to concentration, accumulation also varied with both the method and time of exposure that is the plant growth stage at which rare earths were applied. Thus, under experimental conditions, a fast transport from the root to the tops occurred, which was particularly observed at low dose application. It has been reported earlier that growth enhancement and increase in dry weight of plants after low concentrations of rare earths were applied, are pronounced under extreme environmental conditions.

The root and shoot ratio of *Fesluca arundinacea* increased with the increase **of** La concentration. Effect of La on biomass of *Festuca arundinacea* however, under low concentration was still higher. There was a promoting effect to the growth and development *of Festuca arundinacea* seedlings by dealing with La solution, especially to the growth and development of roots. It could also enhance the control effect against soil-transmitted pathogen and capacity of absorbing nutrients. La could increase the chlorophyll content of the leaves, promote photosynthesis and improve growth and development. Those effects increased with the increase of La concentration and the treatment increased the emergence rate of seedling and promoted the growth and development, especially for the quantity, biomass and absorbing area of roots (Liu et al., 2007).

However, the photosynthetic rate cannot only be increased by mixtures of rare earths but also by single rare earth elements. Accordingly, sole application of cerium also increased chlorophyll contents and photosynthetic rate in spinach. The principle of their improvements on plant photosynthesis is probably related to enhanced enzyme activity, chloroplast development as well as increased chlorophyll contents. Fashui et al. (2002) reported higher contents of chloroplasts and chlorophyll in plants previously treated with cerium. Promoting effects on the photosynthetic rate and intensity as well as on the chlorophyll contents and activity of photosynthetic enzymes are the supportive evidence for increased crop yield and quality reported in several plant varieties due to rare earth application.

The inhibitory activity or toxicity of La to pathogenic fungi is as strong as that of some common organic fungicide used to control the soil-transmitted diseases. Particularly, the toxicity of La to *Rhizoctonia* is even higher than that of fungicides thiram and hymexzol, indicating the potential of La for controlling plant disease. The growth inhibition and abnormal development of mycelium will decrease or destroy its ability to infect and damage plants. The enzyme activity of the fungus also would be inhibited because of the replacement or antagonism of necessary metal ion in pathogenic biological molecule by RE ions. This coordination would modify the structure or configuration of the mycelium and, thereby destroy their function (Mu et al., 2006).

REE would modify the structure or configuration, thereby destroying the function of fungi particularly, the inheritance was perhaps changed when nucleic acid coordinated with RE ions. In general, the inhibitory effect

of La on pathogenic fungi is possibly completed through multisite aggression that alters the enzyme activity and the metabolism of substance and energy (Hui and Weidong, 2002). Chinese scholars and researchers consider that there are correlations between pathogenicity and the ability of the pathogen to produce pectinase based on the fact that pectinase can rapidly degrade host cell and cell wall component, which results in the disease in the host. The number of mycelium and the polygalacturonase activity is reduced after the application of $Nd^{3+}$ (Zhang et al., 2007).

Thus in the present study it was concluded that the rare earth elements not only improve plant growth and disease resistance, could also change the metabolism of plants and disturb the infection process and pathogenicity of the *Aspergillus niger*. Therefore the REEs can be used effectively to control the crown rot disease in *Arachis hypogaea.* Lanthanum, Cerium and Neodymium reduces the deposition of fungal mat on the apex of plant roots, also drastically reduces the enzymatic activities of the *Aspergillus niger.*

Table 6: Pectinase and cellulase activity of the isolated *A.niger* strains

| S. No. | Isolates | Pectinase* | | Cellulase* | | Percentage of Infection | |
|---|---|---|---|---|---|---|---|
| | | With REE | Without REE | With REE | Without REE | With REE | Without REE |
| 1. | M1 | 49.7±0.79 | 53.8±0.81 | 24.7±0.33 | 26.9±0.41 | 29.7±0.04 | 39.5±0.06 |
| 2. | M2 | 38.7±0.70 | 42.5±0.65 | 22.5±0.23 | 28.5±0.40 | 19.8±0.02 | 19.8±0.04 |
| 3. | M3 | 45.7±0.73 | 59.1±0.87 | 26.5±0.42 | 34.2±0.51 | 19.5±0.02 | 28.7±0.05 |
| 4. | M4 | 51.4±0.85 | 55.5±0.76 | 21.5±0.36 | 28.8±0.38 | 9.6±0.01 | 19.6±0.02 |
| 5. | M5 | 29.4±0.42 | 61.2±0.88 | 23.3±0.31 | 29.2±0.33 | 19.7±0.03 | 29.8±0.02 |

*Enzyme activity expressed as percent reduction in viscosity
Values are mean ± SD of triplicates

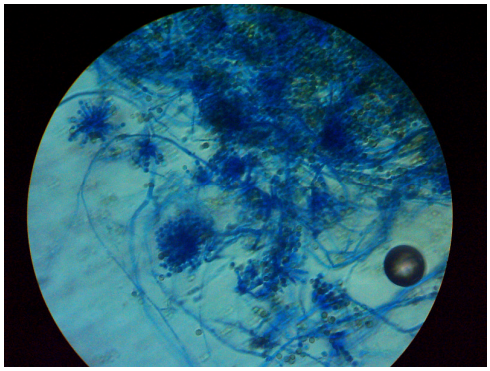

Fig.1. Microscopic observation of *Aspergillus niger* with Lactophenol cotton blue stain

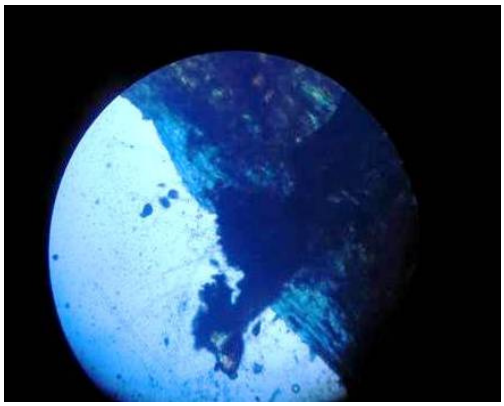

Fig.2. Microscopic observation of infection threads of *A.niger* in groundnut plants

## REFERENCES

[1] H.H. Arts and P.L. Marks "A Summary Table of Biomass and Net Annual Primary Production in Forest Ecosystem". (Ed. Young, H.E.) Life science and Agriculture Experimentation station. University of Marine. Orano, Maire. U.S.A. 1971

[2] S. Bai and X.M. Deng (1995) "Studies on the resistance against cotton blight by rare earth and yield increase effect". *Journal of South West Agricultural University (In China)* 1995,17(1), 28.

[3] H.L. Barnett, "Illustrated genera of imperfecti fungi". 1960,Minneapolis S.N; 225pp

[4] H.Dong and Y. Cohen "Induced resistance in cotton serdlings against fusarium wilt by dried biomass of *Penicilliurn chrysogenurn* and its water extract" 2002, *Phytoparasitica*, 30( 1 ) , 77.

[5] H Fashui, W. Ling, M. Xiangxuan, W.Zheng, and Z. Guiwen "The Biological Trace effect of cerium(III) on the chlorophyll formation in spinach". *Journal Element Research*. 2002, 89(3),263-276.

[6] I.A.S. Gibson "Crown rot, a seeding disease of groundnut caused by *Aspergillus niger*. II. An anamalous effect of organo-mercurial seed dressings." *Trans.Brit.Mycol.Soc.* 1953, 36,112-119.

[7] M. Giovannetti and B. Mossae "An evaluation of technique to measure VAM infection in roots." *New Phytol* 1980, 84,489-500.

[8] Y. He and L. Xue *J. Applied Ecology (Chinese),* 2005,16(10), 1983-1989.

[9] S.J. Hill, T.A. Arowolo, O.T. Butler, S.R.N. Chenery, J.M.Cook, M.S.Cresser, and D.L. Miles "Atomic spectrometry update. Environmental analysis." *J. Anal. At. Spectrom.* 2002, 17, 284–317.

[10] Z. Hiu and H. Weidong "Gene expression analysis of liver in La $(NO_3)_3$ treated rats." World *Journal of Gastroenterology*, 2002.

[11] M. Jarvan (2002) "Rare earth elements affecting the biological processes and yielding abilities of cultivated crops". *Estonian research institute of Agriculture*.2002.

[12] R.Jeya, G. Balasubramanian, and P.K. Thampi "Determination of rare earth elements in Indian coastal monazite by ICP-AES and ICP-MS analysis and their geochemical significance." *Current Science*, 2008, 94,1296-1302.

[13] R. Keureni, M. Shimoni, and Z. Karchi "Peroxidase activity as a biochemical marker for resistance of muskmelon to *Pseudoperonospora cubensis" Phytopathology*,1992, 82, 749.

[14] Y. Liu, Y. Wang, F.Wang, Y. Liu,, J. Cui, L.Hu, and K. Mu "Control effect of lanthanum against plant disease". *Journal of Rare Earths*, 2008, 26, 115-120.

[15] C.P. Mallik and M.B. Singh " Plant enzymology and histoenzymology. A text manual" Kalyani Publishers, Delhi, India,1980, pp 59–60.

[16] K.G. Mu, Ch. Zhang X.Q. Zhao, J.Y .Cui, W.J . Zhang and L.Hu "Effect of lanthanum on mycelium growth and some pathogenic factors." *Journal of Rare Earths,* 2006, 24(4), 485.

[17] R. Parthasarathy, H.B. Desai and S.R. Kayasth "Radiochemical neutron activation analysis of individual rare earth elements in monazite from different geological environments." *J.Radioanal.Nucl.Chem.Lett.* 1986, 105, 277-290.

[18] M. Reuveni and K. Keuveni "Efficacy of foliar application of phosphate in controlling powdery mildew fungus on fieldgrown winegapes : effects on cluster yield and peroxidase activity in berries". *J. Phytopathol*, 1995, 143, 21.

[19] C. Riegeland and J.P. Note "Chicken litter soil amendment effects on soilborne microbes and Meloidogyne incognita on cotton." *Plant Disease*, 2000, 84, 1275.

[20] Z.Wang, D.Liu, P. Lu, and C. Wang "Accumulation of Rare Earth Elements in Corn after Agricultural Application". *Journal of Environmental Quality,* 2001b, 30,37 – 45.

[21] B. Wen, D.A., Uan, X.Q. Shan, F.L. Li and S.Z. Zhang (2001) " The influence of rare earth element fertilizer application on the distribution and bioaccumulation of rare earth elements in plants under field conditions." *Chemical Speciation and Bioavaiability*, 2001,13, 39-48.

[22] F.H.Witham, B.F. Blaydes and R.M. Devlin "Experiments in plant physiology", Van Nostrand Reinhold, New York, USA, 1971, pp 167-200.

[23] X. Xu, W. Z. Zhu, Wang, and G.J. Witkamp "Accumulation of rare earth elements in maize plants (Zea mays L.) after application of mixtures of rare earth elements and lanthanum. *Plant and Soil,* 2003, 252(2), 267 – 277.

[24] Y.F. Zhang, L.F. Yang, K. Chen, and L. Dong "Effects of Neodymium on Growth, Pectinase Activity and Mycelium Permeability of *Fusarium oxysporum". Journal of Rare Earths* , 2007**,25**, 100 – 105.