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EFFECT OF N-ACETYL PYRIMETHANIL AND PYRIMETHANIL COMPOUNDS ON CELLULASE, ENDO ١،٤B GLUCANASE, CHITINASE AND LYTIC ENZYMES PRODUCTION BY *ASPERGILLUS NIGER.*

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ABSTRACT

Two series of pyrimidine derivatives (pyrimethanil and N-acetyl pyrimethanil) were tested to show their effect on the metabolic activity of *Aspergillus niger.* The two compounds have been used with different concentrations of ٠, ٢،٥, ٥ and ١٠ µg/ml to indicate the fungus ability to produce cellulase, Endo ١،٤B glucanase and chitinase enzymes. The highest levels of Endo ١،٤B glucanase and cellulase enzymes production were at concentration of ٢،٥ µg/ml of pyrimethanil compound and ١٠ µg/ ml of N-acetyl pyrimethanil compound. On the other hand, there is no production of chitinase enzyme by *A. niger* was recorded either on the presence or absence of any concentrations of the two compounds. When examining the effect of the two compounds on lytic enzymes production by *A. niger*, the results showed that the concentration of ٥ µg/ml and ١٠ µg/ml of pyrimethanil compound led to increases in enzymes production and their activities on living and killed cells of *E. coli* and *B. subtilis*, respectively. Also by using the N-acetyl pyrimethanil compound with concentrations of ٢،٥ µg/ml and ١٠ µg/ml, led to an increase in the activity of lytic enzymes on living and killed cells of both *E. coli* and *B. subtilis*, respectively. While those lytic enzymes weren't showed, in the presence or absence of the two compounds, any lytic activity on living and killed cells of *A. niger.*

Addition of ٢،٥ µg/ml of pyrimethanil compound and concentration of ١٠ µg/ml of N-acetyl pyrimethanil compound, increased the extracellular protein production by *A. niger*.

Keywords: Pyrimidine derivatives; cellulase; Endo ١،٤B glucanase; chitinase; lytic enzymes; *Aspergillus niger.*

INTRODUCTION

Pyrimidines are heterocyclic compounds with a ring structure of four carbon and two nitrogen atoms ($C_٤H_٤N_٢$). Pyrimidine has many properties in common with pyridine, as the number of nitrogen atoms in the ring increases the ring pi electrons and become less energetic and electrophilic aromatic substitution that gets more difficult while nucleophilic aromatic substitution gets easier (Forster and Staub, ١٩٩٦ and Liu, ٢٠٠٠). Pyrimidines have a long distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of acquired immunodeficiency syndrome (AIDS) (Jain *et al.,* ٢٠٠٦). Pyrimidines and their derivatives are considered to be important for drugs and agricultural chemicals. Pyrimidine derivatives possess several interesting biological activities such as antimicrobial, antitumour, antifungal, antibacterial and

anticancer (Karale and Gill, ٢٠٠٢ and Fathalla *et al.,* ٢٠٠٩). The biological significance of the pyrimidine derivatives taken from that the pyrimidine is a basic nucleus in DNA & RNA of living organisms (Ghoneim and Youssef, ١٩٨٦). The antimicrobial activity of six synthesized pyrido pyrimidine carboxylate derivatives has been recorded by Reddy *et al.,* (٢٠١١), against gram positive bacteria e.g. *Staphylococcus aureus, Bacillus cereus,* gram negative bacteria e.g. *Escherichia coli, Pseudomonas aeruginosa* and fungi e.g. *Aspergillus niger* and *Candida albicans.* Moreover, Chikhalia and Naik (٢٠٠٧) synthesized pyrimidines with good antibacterial activity. Few of them had moderate antibacterial activity. The pyrimethanil compound is well known as a fungicide and has been used as an effective material of many pesticides (Kanetis *et al.,* ٢٠٠٧). *Aspergillus niger* has a strong pathogenic effect on human, animal and plant (Tunev *et al.,* ١٩٩٩). In plants, *A. niger* appears as ablack molds on the fruits or the plant. However, in human and animal, it targets mainly the lung and the respiratory tract causing Aspergillosis (Roehrl *et al*., ٢٠٠٧). In the current research two new main pyrimidine derivatives (pyrimethanil and N-acetyl pyrimethanil) will be tested for their effect on production of cellulase, Endo ١،٤B glucanase, chitinase and lytic enzymes by *Aspergillus niger*.

MATERIALS AND METHODS

Materials

Organic compounds

The two new synthesized organic compounds used in this investigation were kindly provided by Dr. M. A. Waly, Chemistry Department, Faculty of Science (Damietta), Mansoura University. The organic compounds are: pyrimethanil and N-acetyl pyrimethanil.

Microorganisms

All used micro-organisms in this investigation were kindly provided by Dr. M. I. Abou Dobara, Botany Department, Faculty of Science (Damietta), Mansoura University. These local microorganisms include, *Aspergillus niger, Escherichia coli* and *Bacillus subtilis.*

Growth Media

Fungal growth and production media

Aspergillus niger was cultured on Czapek's-agar medium that has the following composition: ٣٠ g. sucrose, ٣ g. $NaNO_3$, ١ g. K_2HPO_4, ٠,٥ g. $MgSO_4.7H_2O$, ٠,٥ g. KCl, ٠,٠١ g. $FeSO_4.7H_2O$, ١٥ g. agar and ١ litre of distilled water. The pH was then adjusted to ٦,٥ using ٠,١ N NaOH. The media were used for production after addition of pyrimethanil and N-acetyl pyrimethanil compounds and inoculated with spore suspension of *A. niger.* After ٧ days of incubation at ٣٠ ° C, culture was filtrated and culture filtrate used as enzyme source.

Bacterial growth medium

Escherichia coli and *Bacillus subtilis* were cultured on nutrient-agar medium that has the following composition: ١٠ g. peptone, ٣ g. beef extract, ١٧ g. agar and ١ litre of distilled water, and the pH was adjusted to ٧,٢ using ٠,١ N NaOH.

Methods

Preparation of colloidal chitin:

Colloidal chitin was prepared by treating the forgoing material with acetone to form a paste, then ٥-١٠ volumes of conc. HCl was added slowly while grinding in a morter with the temperature maintained at ١٠ – ٢٠˚C to arrest hydrolysis. After several minutes, the syrupy liquid was filtered through glass wool, and poured into vigorously stirred ٥٠٪ aqueous ethanol to precipitate the chitin in a highly dispersed state. The residue was sedimented and resuspended in water several times to remove excess acid alcohol, then dialyzed against tap water.

Assay of cellulase, glucanase and chitinase:

Enzyme activity was determined colourimetrically where one ml of culture filtrate was added to ١ ml of ٠,٠٥ M citrate buffer, pH ٤,٨, containing ١٠ mg of substrate (crystalline cellulose, carboxymethylcellulose and colloidal chitin, respectively). After incubation for ٣٠ minutes at ٤٠˚C, the reaction was terminated and reducing sugar released was determined by the dinitrosalicylic acid method (Miller, ١٩٥٩). The tubes were placed in a boiling water bath for ١٥ min, then cooled to room temperature and the absorbance was measured at ٥٥٠ nm. Known concentrations of glucose (١ – ٥ µmole/ml) were used in the same manner to construct the standard curve of glucose. One unit of enzyme activity was defined as the ability of producing the reducing sugars equivalent to ١ µmole of glucose per minute.

Lytic Activity of Enzyme Preparation from *Aspergillus niger:*

A. niger was routinely grown on Czapek's-agar medium and subcultured whenever required. Triplicate sets of ٢٥٠ ml Erlenmeyer flasks each containing ٥٠ ml of the following medium (g/١٠٠ ml): peptone, ٠,٥; meat extract, ٠,٢; yeast extract, ٠,١; $MgSO_{٤}.٧H_{٢}O$, ٠,٠٥; NaCl, ٠,٥ and sucrose, ١; and the pH was initially adjusted to ٦,٥. The medium was inoculated with ١ ml of spore suspension obtained from ٧-day-old cultures. The mycelial mats were incubated at ٣٠˚C for ٧ days, at the end of incubation the flasks were filtered and the filtrate of each set was mixed and completed with distilled water to ١٥٠ ml. Crude enzyme preparation was precipitated from the culture filtrate using ٦٠٪ saturation of ammonium sulphate overnight at ٤˚C. The precipitate was then redissolved in ٠,٠٥ M phosphate buffer (pH ٦,٤). Measurement of lytic activity was carried out using ١ ml of ٠,٠٥ M phosphate buffer (pH ٦,٤) which contained ٠,٠٦٪ (w/v) of living and killed cells of (*Aspergillus niger, Escherichia coli* and *Bacillus subtilis*), ١ ml of enzyme containing solution was added and the change of absorbance was recorded at ٦٦٠ nm after incubation at ٣٠˚ C for ٣٠ min. One unit of lytic activity was expressed as the amount of enzyme giving an initial decrease in optical density (OD) of ٠,٠٠١ per minute (Ghareib and Nour El Dein, ١٩٩٤).

Protein profile and determination of total protein of *Aspergillus niger:*

***Aspergillus niger* extract preparation:**

Fungal culture was filtered and the mat was collected, washed twice with distilled water, and ١٠ mM Tris-HCl buffer (pH ٦,٠) and subjected to homogenization.

Homogenization:

١٠٠ g. of the biomass of *Aspergillus niger* were homogenized in ٢٠٠ ml of the same buffer. The homogenate was filtered through cheese cloth then centrifuged at ٤٠٠٠ r. p. m. for ٥ minutes and the resulting supernatant was used.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Protein profile was done by using one-dimensional polyacrylamide gel electrophoresis according to the method of Laemalli (١٩٧٠).

Determination of Total protein:

The proteins were determined according to the method of Lowry *et al.,* (١٩٥١) using bovine serum albumin solution with concentration ٥٠ mg% as standard protein.

RESULTS

Effect of pyrimethanil compound on cellulase, Endo ١،٤B glucanase and chitinase enzymes production by *A. niger:*

Figure ١ indicates the relation between the pyrimethanil compound concentration and the production of cellulase, Endo ١،٤B glucanase, and chitinase by *A. niger*. It was clear from the first glance that both cellulase and Endo ١،٤B glucanase gradually increased till their maximum level above ٢,٥ U/ml and ١ U/ml, respectively as the concentration level of pyrimethanil compound became high till a certain concentration (٢,٥ µg/ml). A sharp decline of cellulase and Endo ١،٤B glucanase was observed when the concentration of pyrimethanil compound became high, then the level of cellulase and Endo ١،٤B glucanase production stay at the same level about ٠,٦ U/ml and ٠,٥ U/ml, respectively with no change even with elevation in the pyrimethanil compound concentration. On the other hand the ability of *A. niger* to produce chitinase was zero with different used concentrations of the pyrimethanil compound.

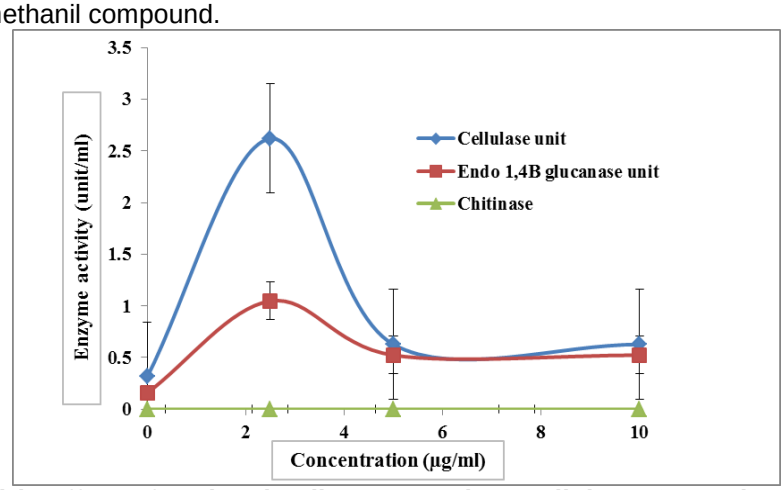

Fig. (١): Effect of pyrimethanil compound on cellulase, ١،٤B glucanase and chitinase enzymes production by *A. niger*.

Effect of N-acetyl pyrimethanil compound on cellulase, Endo ١،٤B glucanase and chitinase enzymes production by *A. niger:*

Figure ٢ indicates the relation between the N-acetyl pyrimethanil compound and the ability of *A. niger* to produce cellulase, Endo ١،٤B glucanase, and chitinase. It is clear that the production of Endo ١،٤B glucanase was slightly higher than cellulase enzyme at zero concentration of pyrimethanil compound. A gradual decrease of cellulase and Endo ١،٤B glucanase was recorded until an inflection occurred at ٢,٥ µg/ml concentration. When the concentration of the N-acetyl pyrimethanil compound is increased a gradual rise of the amount of the cellulase and Endo ١،٤B glucanase were observed until concentration reached to of ٥ µg/ml, and after this a high elevation in the level of both enzymes was noticed. At high concentration of N-acetyl pyrimethanil compound (١٠ µg/ml), cellulase and Endo ١،٤B glucanase production increased to ٢,٤ U/ml and ١,٨ U/ml, respectively. With regard to the chitinase level, there was no change, and its level stayed zero even when the concentration of N-acetyl pyrimethanil was increased to ١٠ µg/ml.

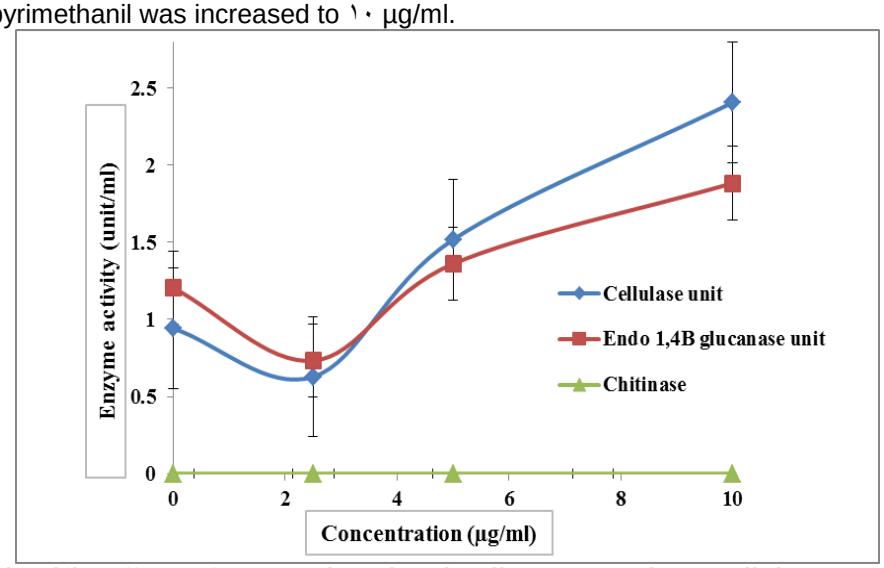

Fig. (٢): Effect of N-acetyl pyrimethanil compound on cellulase, ١،٤B glucanase and chitinase enzymes production by *A. niger*.

Effect of pyrimethanil compound on lytic enzymes production by *Aspergillus niger* and their activity on living cells of *Escherichia coli, Bacillus subtilis* and *Aspergillus niger:*

Figure ٣ shows the relation between the pyrimethanil compound concentration and the activity of lytic enzymes produced by *A. niger* on living cells of *B. subtilis, E. coli* and *A. niger*. The activity of lytic enzymes produced by *A. niger* on living cells of *E. coli* and *B. subtilis* were ٢٣% and ٧%, respectively at zero concentration of pyrimethanil compound. With increasing the concentration of pyrimethanil compound, a dramatic fall dawn of the

activity of lytic enzymes produced by *A. niger* on *E. coli* was observed. It became dominant till concentration of an approximate ٢,٥ µg/ml. A huge drop of the activity of lytic enzymes produced (٣,٥ %) by *A. niger* occurred when the concentration of pyrimethanil compound was increased to ١٠ µg/ml. But with additional increase of pyrimethanil compound concentration up to ٥µg/ml, the activity of lytic enzymes produced by *A. niger* on *B. subtilis* was increased and reached to its maximum level (١٦.٥٪). The activity of lytic enzymes produced by *A. niger* on their cells showed no change even when more concentrations were used and the level of lytic enzyme activity stay at zero line trend.

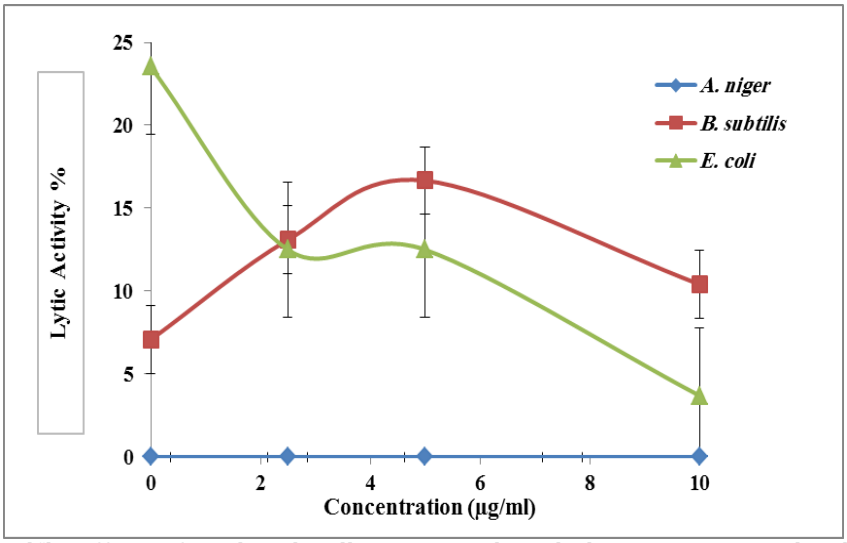

Fig. (٣): Effect of pyrimethanil compound on lytic enzymes production by *A. niger* and their activity on living cells of *E. coli*, *B. subtilis* and *A. niger*.

Effect of pyrimethanil compound on lytic enzymes production by *Aspergillus niger* and their activity on killed cells of *Escherichia coli*, *Bacillus subtilis* and *Aspergillus niger*:

Figure ٤ shows the percentage of lytic enzymes production by *A. niger* and their activity on killed cells of *B. subtilis*, *E. coli* and *A. niger* and concentration of pyrimethanil compound. The activity of lytic enzymes produced by *A. niger* on *E. coli* showed fluctuation up and down as the concentration of pyrimethanil compound gradually increased from zero to ١٠ µg/ml where ٢,٥ µg/ml and ١٠ µg/ml show maximum level of the activity of lytic enzymes produced by *A. niger* ١٢,٩٪. While on *B. subtilis* the lytic enzymes activity had a slight effect as the concentration of pyrimethanil compound increased to ١٠ µg/ml. The activity of lytic enzymes produced by *A. niger* on their cells showed no effect when different concentrations of pyrimethanil compound were used.

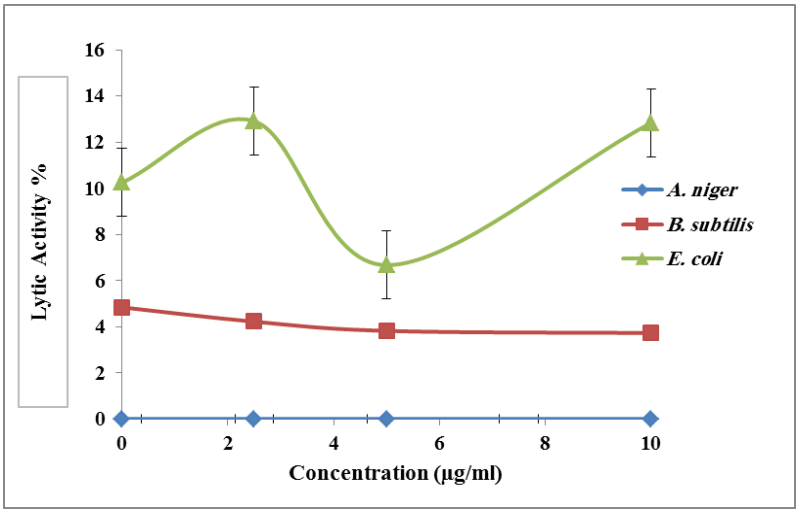

Fig. (٤): Effect of pyrimethanil compound on lytic enzymes production by *A. niger* and their activity on killed cells of *E. coli*, *B. subtilis* and *A. niger*.

Effect of N-acetyl pyrimethanil on lytic enzymes production by *Aspergillus niger* and their activity on living cells of *Escherichia coli*, *Bacillus subtilis* and *Aspergillus niger*:

Figure ٥ indicates the effect of N-acetyl pyrimethanil on lytic enzymes production by *A. niger* and their activity on living cells of *E. coli*, *B. subtilis* and *A. niger*. The activity of lytic enzymes produced by *A. niger* on living cells of *E. coli* and *B. subtilis* were increased to a certain range ١٦ % and ١٠,٥ %, respectively at ٢,٥ µg/ml and then a marked reduced had happened to ١٣ % and ١٠ %, respectively at ٥ µg/ml in both bacteria. Different trends of the activity of lytic enzymes produced had been taken as the concentration increased from ٥ µg/ml to ١٠ µg/ml. The activity of lytic enzymes produced by *A. niger* on *B. subtilis* reached to its maximum level (١٦ %) at ٢.٥ µg/ml but ١٧,٥ % was recorded at ١٠ µg/ml on *E. coli.* On the other hand the lytic enzymes activity produced by *A. niger* on living cells of *A. niger* had not been affected even with increasing the concentration of N-acetyl pyrimethanil from zero to ١٠ µg/ml concentration, and still fixed with the zero value.

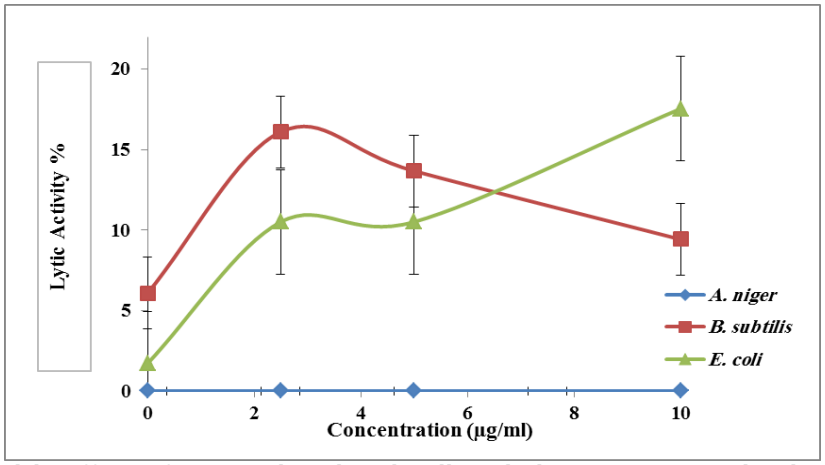

Fig. (٥): Effect of N-acetyl pyrimethanil on lytic enzymes production by *A. niger* and their activity on living cells of *B. subtilis, E. coli* and *A. niger*.

Effect of N-acetyl pyrimethanil on lytic enzymes production by *Aspergillus niger* and their activity on killed cells of *Escherichia coli, Bacillus subtilis* and *Aspergillus niger:*

Figure ٦ shows the relation between concentration of N-acetyl pyrimethanil compound and the activity of lytic enzymes produced by *A. niger* on killed cells of *B. subtilis, E. coli* and *A. niger*. The activity of lytic enzymes produced by *A. niger* on *E. coli* fluctuated as the concentration of the compound gradually increased from zero to ١٠ µg/ml and reached its maximum level (١٢ %) at ١٠ µg/ml, while the activity of lytic enzymes produced slightly increased on *B. subtilis* as the concentration increased from zero to ١٠ µg/ml. Finally the activity of lytic enzymes produced by *A. niger* on killed cells of *A. niger* was not changed, where zero scale of lytic enzyme level of *A. niger* was recorded.

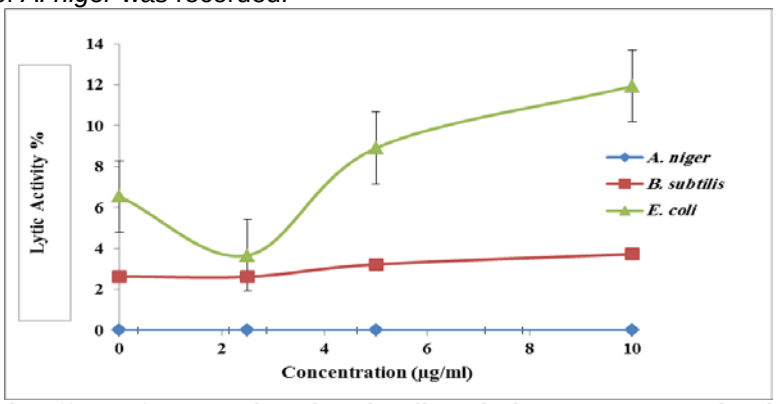

Fig. (٦): Effect of N-acetyl pyrimethanil on lytic enzymes production by *A. niger* and their activity on killed cells of *B. subtilis, E. coli* and *A. niger*.

Effect of pyrimethanil and N-acetyl pyrimethanil compounds on extracellular total protein production by *A. niger:*

Figure ٧a & ٧b indicates the effect of pyrimethanil and N-acetyl pyrimethanil compounds on the total protein production by *A. niger*, respectively. It can be seen from figure ٧a that the percentage of the total protein was sharply increased as the amount of the concentration of pyrimethanil rises up and reached to its maximum level at ٢,٥ µg/ml then a decline in the total protein level occurred. The N-acetyl pyrimethanil compound had a different effects on the total protein percentage. At concentration of zero the total protein percentage was ١٠٤ mg%. This amount starts to decline as the concentration of N-acetyl pyrimethanil compound increased up to ٥ µg/ml. At this concentration a sharp rises up in the amount of the total protein and reached to its maximum activity at ١٠ µg/ml.

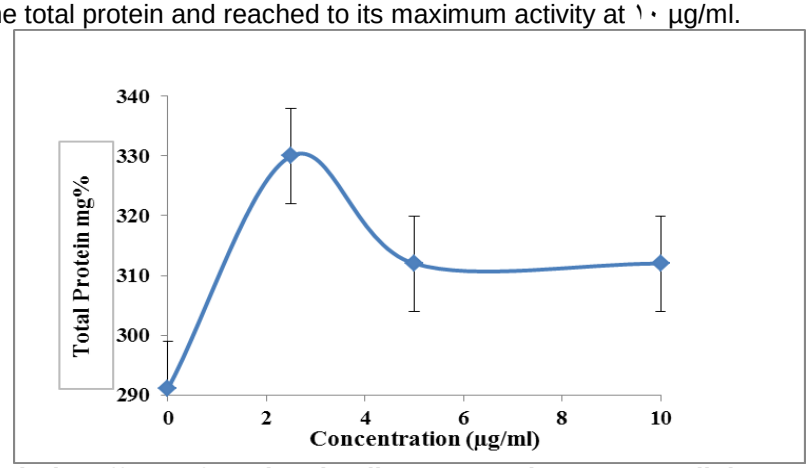

Fig. (٧a): Effect of pyrimethanil compound on extracellular protein produced by *A. niger*.

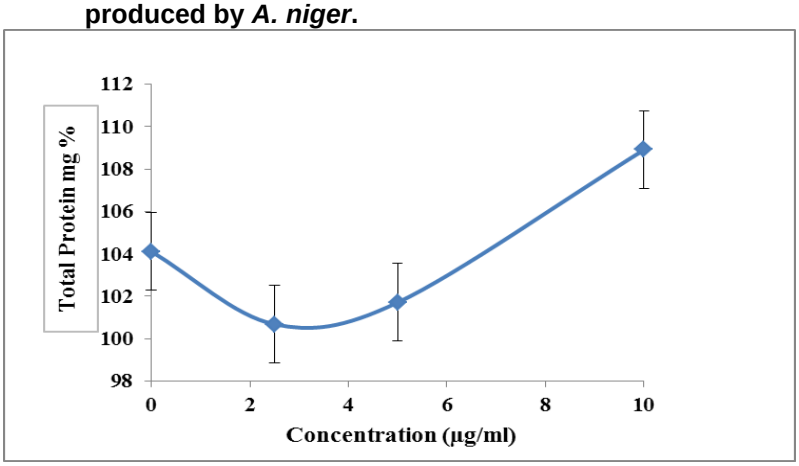

Fig. (٧b): Effect of N-acetyl pyrimethanil compound on extracellular protein produced by *A. niger.*

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis:

The electrophoresis of the proteins of *Aspegillus* niger homogenate was depicted in (Fig. ٨). The effect of pyrimethanil and N-acetyl pyrimethanil compounds with different concentrations (٥ µg/ml and ١٠ µg/ml) against *A. niger* growth was more obviously in the electrophoresis analysis for homogenate (Fig. ٨). In this figure there is a difference between protein patterns of normal *A. niger* (lane ١) and protein patterns of the effect of different concentrations of pyrimethanil and N-acetyl pyrimethanil compounds on *A. niger* (lane ٢, ٣, ٤, ٥)**.**

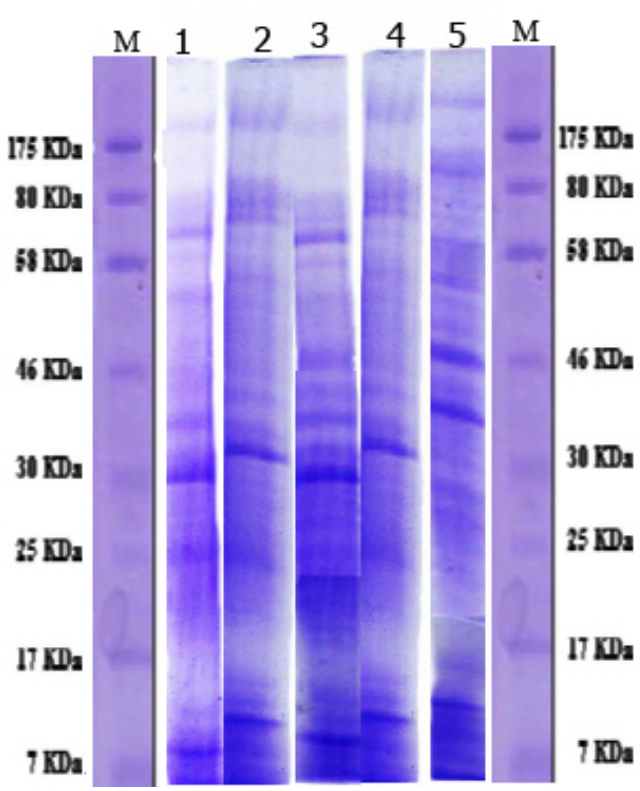

Fig. (٨): Separation of proteins in *Aspegillus niger* homogenate sample by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique at ٢٠٠ V for ١,٥ hr. Lane ١ indicates normal *A. niger*, lane ٢ indicates effect of pyrimethanil compound with concentration of ٥ µg/ml on *A. niger,* lane ٣ indicates effect of pyrimethanil compound with concentration of ١٠ µg/ml on *A. niger,* lane ٤ indicates effect of N-acetyl pyrimethanil compound with concentration of ٥ µg/ml on *A. niger,* lane ٥ indicates effect of N-acetyl pyrimethanil compound with concentration of ١٠ µg/ml on *A. niger,* lane M indicates molecular weight marker.

DISCUSSION

Aspergillus niger has been reported to cause numerous chronic diseases to humans, animals and plants; and has a pathogenic effect called aspergillosis (Tunev *et al.,* ١٩٩٩). *Aspergillus niger* affect plants in the form of black molds, and targets mainly the lung and the respiratory tract in both animals and humans (Roehrl *et al*., ٢٠٠٧); also it may cause otomycosis a serious damage to the ear canal and tympanic membrane (Steinbach and Stevens, ٢٠٠٣). The development of new effective compounds towards the production of cellulase, Endo ١،٤B glucanase, chitinase and lytic enzymes by *A. niger* is required to control the pathogenicity of the fungus *A. niger.* Pyrimidine compound derivatives which used in this work showed an antifungal effect when they was tested against *A. niger.* The pyrimethanil compound has a past record to be known as afungicide and pesticide compound. In this research we introduced for the first time the biological activity of N-acetyl pyrimethanil compound in compared with the standard known compound; pyrimethanil on cellulase, Endo ١،٤B glucanase, chitinase and lytic enzymes production by *A. niger*. Fritz *et al.,* (١٩٩٧) used the Anilinopyrimidine such as Fungicide pyrimethanil to inhibit the growth of fungus *Botrytis cinerea*, and indicated it as antifungal agent, which can be able to act as an important antiplant pathogen.

The Effect of pyrimethanil compound on cellulase, ١،٤B glucanase and chitinase enzymes production by *A. niger* has been determined. Both levels of cellulase, Endo ١،٤B glucanase were affected by the amount of the pyrimethanil compound and reached to their maximum level at ٢.٥ µg/ml. No chitinase was detected at all. Our results regard to the effect of pyrimidine derivative on lytic enzyme production by *A. niger* from one hand is disagreed by Kishore *et al.,* (٢٠٠٦) who treated the bacterial cells with Anilinopyrimidine (pyrimethanil) and indicated that a rapid accumulation of defense-related enzymes like chitinase, which remains undetectable in our work.

On the other hand, ١٠ µg/ml of the N-acetyl pyrimethanil compound increased cellulase and Endo ١،٤B glucanase production by *A. niger.* No chitinase was detected at all even with increase in the concentration of the N-acetyl pyrimethanil compound. The effect of N-acetyl pyrimethanil compound is mostly confirmed by Kishore *et al.,* (٢٠٠٦) who reported that there is an accumulation of both cellulase and glucanase enzymes were increased as a result of the bio-control activity of N-acetyl pyrimethanil compound in inhibition of fungal cell wall-degrading enzymes.

Effect of pyrimethanil compound on lytic enzymes production by *A. niger* and their activity on living cells of, *B. subtilis, E. coli* and *A. niger* was investigated. *A. niger* showed low lytic enzymes activity on *E. coli* as the concentration of pyrimethanil compound increased (١٠ µg/ml) and high lytic enzymes activity on *B. subtilis* as the concentration of pyrimethanil compound increased to ٥ µg/ml. On the other hand the activity of lytic enzymes produced by *A. niger* on their living cells was zero. This result is come in line with Akcelik and Tuckel (٢٠٠٣) who tested the antimicrobial activities of some pyrimidine derivatives and indicates that inhibitory effect against different indicator bacteria in living cells is increased in the presence of substrates.

Increasing the concentration of N-acetyl pyrimethanil compound from zero concentration to ١٠ µg/ml in the presence of *B. subtilis, E. coli* and *A. niger* acted with different behaviors. *A. niger* produced high levels of lytic enzymes and showed high lytic enzymes activity as the concentration of pyrimethanil compound increased to ١٠ µg/ml on *E. coli* and ٥ µg/ml on *B. subtilis*. With regard to the activity of lytic enzymes produced by *A. niger* on their living cells, there is no effect present as the concentration of N-acetyl pyrimethanil compound increased.

Also we can recognize that the activity of lytic enzymes produced by *A. niger* on *E. coli* showed fluctuation up and down as the concentration of pyrimethanil compound gradually increased from zero to ١٠ µg/ml, while on killed cells of *B. subtilis* it had a slight decrease. The lytic enzymes activity produced by *A. niger* on killed cells of *A. niger* stayed at zero at all used concentrations. This work also has been confirmed by Akcelik and Tuckel (٢٠٠٣) who tested the antimicrobial activities of some pyrimidine derivatives and indicates that inhibitory effect against different indicator bacteria in killed cell was increased in the presence of substrates.

The relation between concentration of N-acetyl pyrimethanil compound and the activity of lytic enzymes produced by *A*. *niger* on killed cells of *B. subtilis, E. coli* and *A. niger* was observed. The activity of lytic enzymes produced by *A. niger* on *E. coli* fluctuated as the concentration of the compound gradually increased from zero to ١٠ µg/ml, while a slight increase in the activity of lytic enzymes produced on *B. subtilis* had occurred. The activity of lytic enzymes produced by *A. niger* on killed cells of *A. niger* was not changed and stayed at the started zero points as an additional concentration be added.

The percentage of the extracellular protein produced by *A. niger* was affected by the two compounds, where ٢,٥ µg/ml of pyrimethanil and ١٠ µg/ml of N-acetyl pyrimethanil increased total protein production by *A. niger.* This result is confirmed by Milling and Richardson (١٩٩٥), who estimated the effect the anilino-pyrimidine such as fungicide pyrimethanil on the total protein levels in *Botrytis cinerea* and indicates rapid increase in the total protein levels.

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تأثير مركبات ان استيل بريميثانيل والبريميثانيل على إنتاج السليلوليز، اندو١ ،٤ بيتا جلوكانيز، الكيتينيز وانزيمات التحلل بواسطة اسبرجلس نيجر

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تم اختبار مركبين اساسين من مشتقات البريميدين (البريميثانيل وان أسيتيل بريميثانيل) على النشاط الأيضى للاسبرجلس نيجر حيث تم دراسة تأثير المركبين بتركيزات مختلفة ٠ ، ٢,٥ ، ٥ و ١٠ ميكروجرام/مل على انتاج الفطر لانزيمات الكيتينيز، الجلوكانيز والسليلوليز . وكانت أعلى زيادة فى انتاج انزيمات الجلوكانيز والسليلوليز باضافة تركيز ٢,٥ ميكروجرام/مل من مركب البريميثانيل و ١٠ ميكروجرام/مل من مركب ان اسيتيل بريميثانيل. ومن ناحية اخرى، فإن انزيم الكيتينيز لم يتم انتاجه من الفطر سواء فى وجود او عدم وجود أى تركيزات من هذين المركبين.

وعند دراسة التأثير على انتاج الفطر لانزيمات التحلل أظهرت النتائج ان تركيز ٥ ميكروجرام/مل و ١٠ ميكروجرام/مل من مركب البريميثانيل أدت الى زيادة انتاج هذه الانزيمات ونشاطها فى تحليل الخلايا الحية والميتة من ميكروبى ايشيريشيا كولاى و باسيليس ستيليس، على الترتيب. وباستخدام مركب ان اسيتيل بريميثانيل بتركيزى ٢,٥ ميكروجرام/مل و ١٠ ميكروجرام/مل أدى الى زيادة فى نشاط انزيمات التحلل للخلايا الحية والميتة لكل من ايشيريشيا كولاى و باسيليس ستيليس، على الترتيب. بينما لم تظهر انزيمات التحلل المنتجة بالفطر فى وجود او عدم وجود المادتين أى نشاط تحليلى تجاه خلاياه سواء الحية او الميتة.

ولقد أدت إضافة ٢,٥ ميكروجرام/مل من مركب البريميثانيل و تركيز ١٠ ميكروجرام/مل من مركب ان اسيتيل بريميثانيل الى زيادة انتاج البروتينات الكلية فى البيئة بواسطة فطر الاسبرجلس نيجر.

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