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EFFECT OF FOUR PESTICIDES ON VIABILITY AND PRESENCE OF PLASMIDS IN TWO RHIZOBIUM JAPONICUM STRAINS

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ABSTRACT: *We used two Rhizobium Japonicum strains 1577 and R.J.G as preventatives to investigate pesticides deleterious effects on soil bioflora. We tested bacterial survival in presence of four tested pesticides Methomyl, Monocrotophos, Tralomethrin and Abamectin at 1/200 of LD50 of each.*

Furthermore, we prepared DNA plasmid out of bacterial strains that have been grown for several days in ligued yeast extract mannitol media (YEM).

Our results showed that bacterial strain 1577 has higher ability to survive in presence of tested pesticides with varying degrees ranging from 0.50 to 0.867%.

Test pesticides cause loss of nif and/or fix plasmids that ordinary involved in nitrogen fixation in their symbiotic relationship with legumes. Therefore care should be taken not to use those pesticides frequently to reduce the level of ecosystem pollution.

Key words: *pesticides, plasmids, rhizobium, japonicum.*

INTRODUCTION

Agriculture practices are both cause and victim of environmental pollution. Pesticides are compos term that includes all chemicals in agriculture. At the same time it is prime source of environmental pollution. This is mainly due to inadequate, inappropriate and excessive use. The ecological effects of pesticides at the microorganism's level are usually considered to be an early warning indicator of potential human health impacts (Whitmore, 1996). Members of the genus Rhizobium are of great economic atmospheric importance because of their ability to fix nitrogen (Parakash and Altherly 1986). They fix nitrogen symbiotically with legumes (Baladani *et al* ;1992).

Genes responsible for nitrogen fixation mediate stress response, and synthesis of melanin, lipopolysacharids and becterocim appeared to be present on plasmids. (Maitinez et al; 1990 and Baladani and weaver 1992). We conducted this research to asses dangerous effect (S) of four pesticides on two Rhizobum japonicum strains.

This was mainly done by detecting survivability and presence or absence of fix and/or mod plasmids.

MATERIALS AND METHODS

We used two strains of Rhizobium Japponicum 1577 and R.J.G that were obtained from biological nitrogen fixation unit. Agricultural Research Center, Sakha.

They were grown in yeast extract mannitol (YEM) media (liquid and solid). Four pesticides were used in this study.

They were Methomyl (acute oral LD50 for male rats 34, female rats 30mg/kg) Monocrotophos (acute oral LD50 for male rats18, female rats 20mg/kg) Tralomethrin (acute oral LD50 for rats 99-3000mga.i/kg) and Abamectin (LD50 for rats10, mice13.6mg/kg). Bacterial cultures were diluted several times to give an appropriate number of cells. Pesticides were added at 1/200 of LD50 to the growing media. Each was replicated four times. Number of growing colonies were recorded and analyzed statistically.

Furthermore, plasmids were prepared from tested bacterial strains that have been grown for several days in liquid YEM at 28c. Media were fortified with 1/200 of LD50.

Plasmids preparations were performed according to (Birnboin and Doly 1979) . DNA preparations were subjected to 0.7% agarose gel electrophoresis with ethedium bromide dye. Plasmids were visualized on ultraviolet Tran illuminator and photographed.

RESULTS AND DISCUSSIONS

Genotoxic effects of the four tested pesticides on Rhizobium japonicum strains 1577 and R.J.G are clearly pronounced as indicated in Table (1) by detecting survival percentage that deduced from number of viable cell count.

It appeared that R.J.G strain is more sensitive to all tested pesticides being most sensitive to Abamectin giving rise to 0.738% growth inhibition. This followed by Moncrotophos, Methomyl and Tralomethrin as it recorded 0.705, 0.689 and 0.680 Percentages respectively.

Mean while R.J.G strain 1577 showed more ability to survive in presence of pesticides. It showed the highest value survival 0.867% in presence of Abamectin. Moreover it appeared to survive at 0.62 in presence of Methomyl.

In spite of it survived Monocrotophos and Traomethrin, it showed 50% growth inhibition. It might be helpful to point out that genetic diversity between the two tested Rhizobium strains is the main cause of the above mentioned results toward various chemical pesticides.

These results are in accordance with what Eisa and Eissa (1992) reported using different pesticides on Rhizobium strains

More over, Abd El-Rehim *et al* (1992) reported that the presence of herbicides in clay loam soil gave rise to higher nitrogenase activity due to increasing of Rhizobium activity and this fact goes nicely with what we found using Rhizobium strain R.J.G. However this could be due to variations in pesticides mode of action on soil micro-organisms (Sutter *et al*.1971).

The higher Rhizobium survival rate could be due to their abilities to degrade the pesticides as it mentioned lately by Feng and Kennedy (1997) .

%S Survival percentage of treated cells
% 1 percentage Inhibition of treated cells.
% Of survival cells (%S) = (number of survived cells / total plated number of cells)×100
Number of inhibited cells = total plates number – number of survived
% Of inhibit (%I) = (number of inhibited cells / total number of plated cells)×100

Similar results have been published before (Lee and Banks, 1993) as they found that microbial number in alfalfa *Rhizobium* increased substantially in petroleum contaminated soil.

However, Martinez *et al*. (1990). Found that herbicides decreased and inhibited nitrogen fixation and growth in pure culture of *Azospirllium brasilense* in malate media.

Table (1): Genotoxic effects of tested pesticides on *Rhizobium Japonicum* 1577 and R.J.G.strains.

Pesticides	*Rhizobium Japonicum*			
	Strain 1577		Strain R.J.G.	
	% S	% I	% S	%I
Methomyl	0.620	0.380	0.302	0.689
Monocortophos	0.530	0.470	0.295	0.705
Tralomethrin	0.500	0. 500	0.320	0.680
Abamectin	0.860	0.133	0.262	0.738

We intended in this experiment to illustrate the disbenefits of pesticides that threaten the long term survival of major ecosystem including beneficial microorganism such as *Rhizobia*.

Therefore, we studied the effects of the four tested pesticides polluted soils on the maintenance of two strains of *Rhizobia*. This was done by looking for nit or fix plasmids that ordinary involved in nitrogen fixation of their symbiotic relationship with legumes (Watson *et al*.1988 and Banfalni *et al*.1992).

Data in fig (1) represent a photograph of mini preparations of several *Rhizobial* isolate that allowed to grow in presence of the four tested pesticides lanes 1,2,4,5 and 6 contains DNA of plasmid preparations of strain 1577 that was plates on YEM fortified with Abamectin, Tralomethrin, Methomyl and Monocrotophos respectively.

Moreover lanes 3, 7, 8, and 9 contain DNA repaired isolates of strains R.J.G that was plates in presence of Abamectin, Tralomethrin, Methomyl and Monocrotophos respectively.

Lanes 14 and 15 DNA prepared from bacterial isolates of strain 1577 and R.J.G. that grow in absence of pesticides absent of the plasmids in most tested bacterial isolated. Lower viable cell count in presence of the tested pesticides may be attributed to inability of those isolates to overcome pesticide stress. This fact is in accordance with what Baladani and weaver (1992) suggested that the involvement of some plasmids in *Rhizobia* in mediating the response to the stress conditions.

We have to mention that pesticides may persist in soil for long periods of time.

Therefore frequent applications in agricultural soils may results in accumulations of high amounts of those compounds leading to inhibition of Rhizobium growth and nitrogen fixation as well. Therefore care should be taken in pesticides used to maintain clean environment and reduce the level of ecosystem pollution.

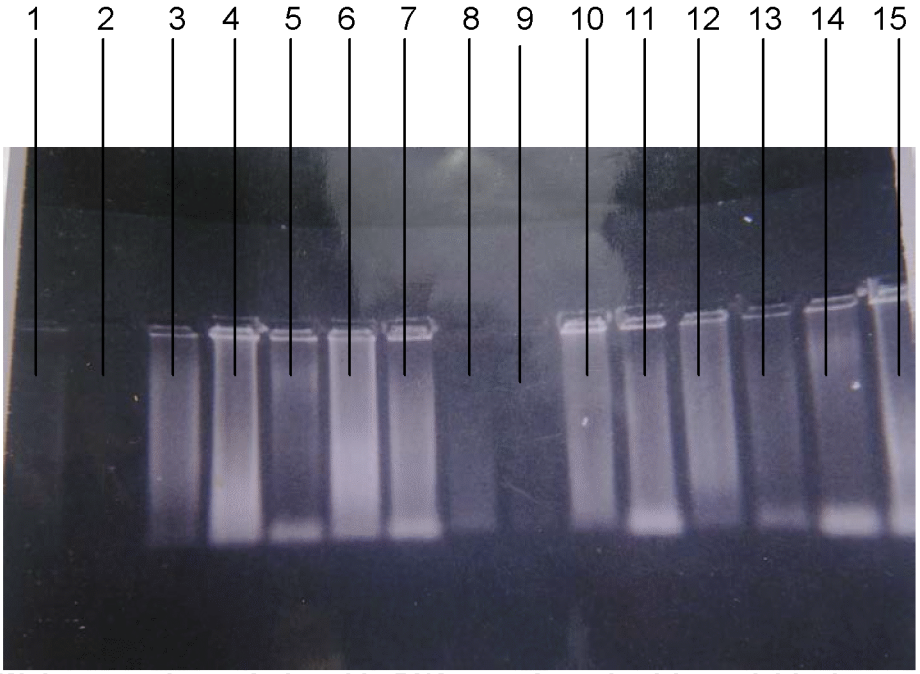

Fig (1): Mini preparations of plasmids DNA out of survived bacterial isolates of strains 1577 and R.J.G.

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تأثير أربعة مبيدات على حيوية ووجود البلازميدات في سلالتين من الرايزوبيوم جابونكم

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الملخص العربى

تم استخدام سلالتين من الرايزوبيم جابنكوم 1577و R.J.G لايضاح مدى تاثير المبيدات الحشريه على الكائنات الدقيقه فى التربه.

تم قياس حيوية البكتريا فى وجود اربعة مبيدات ميزوميل ,مونوكروتوفوس ,ترالوميثرين وأبامكتين.

استخدمت المبيدات الاربعه بتركيز 200/1 من تركيز كلا منهم النصف مميت .

بالاضافه الى ذلك تم تحضير الماده الوراثيه للبلازميده الموجوده فى البكتريا الناميه على بيئه مستخلص الخميره والمانيتول .

اوضحت النتائج المتحصل عليها ان سلاله البكتريا 1577ذات قدره مرتفعه للنمو فى وجود المبيدات الاربعه وذلك بدرجات متفاوته تتراوح من 2/1 الى 87و%.

اثرت المبيدات المستخدمه على وجود البلازميدات فى البكتريا التى تحمل جينات يتم بواسطتها تثبيت الازوت الجوى بعلاقه التكافل مع النباتات البقوليه مما يؤدى الى فقد البكتريا لهذه الخاصية.

وعليه نوصى بالحذر فى استخدام هذه المبيدات بكثافه لتقليل مستوى التلوث البيئى للوسط المحيط.