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NEMATICIDAL POTENTIAL OF SOME ESSENTIAL PLANT OILS AND YEAST EXTRACT IN CONTROLLING *Meloidogyne Incognita* AND *Rotylenchulus Reniformis* ON TOMATO.

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ABSTRACT

The nematicidal potential of clove oil, black cumin oil, orange peels oil, tobacco (2% nicotine) and yeast extract were evaluated for controlling the root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis* infecting tomato plants cv. Balady under greenhouse conditions. All the treated materials significantly reduced number of galls, immature stages, females, egg-laying females and total number of nematodes in tomato roots. The highest significant reduction was recorded in numbers of swollen and egg-laying females of *R. reniformis* on tomato roots. In most cases, the previous materials were more effective on *Rotylenchulus reniformis* than on *Meloidogyne incognita*. Yeast and tobacco extracts gave the greatest reduction on numbers of both species. There was a positive reaction, in most cases, between essential oils, tobacco and yeast extract treatments and tomato plant growth.

Keywords: Essential oils, *Meloidogyne incognita*, nematode, *Rotylenchulus reniformis*, yeast and nicotine extract.

INTRODUCTION

Tomato, *Lycopersicon esculentum* Miller is one of the most important vegetables growing in Egypt and worldwide. The root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood and the reniform nematode, *Rotylenchulus reniformis* (Linford and Oliviera) are the most important and common nematode pests in Egypt (Ibrahim, 1994). Plant parasitic nematodes are controlled by cultural practices, solarization, growing of resistant cultivars and chemical nematicides. However, nematicides are expensive and harmful on health, efforts are needed to develop alternative nematode management strategies, effective, safe and less cost methods of control. Many plants are known to have nematicidal effect against plant parasitic nematodes when they are used as soil amendments or plant extracts. There are many reports on utilization of plant parts as organic amendments for the control of nematodes (e.g. Anjum *et al.*, 1996; Ehteshamul *et al.*, 1996; Rao *et al.*, 1996; Amin and Youssef, 1997 and 1998,Youssef & Amin 1997; Ismail 1998 and Zarina *et al.*, 2003). Other investigators tested the plant as extracts or oils (e.g. Miller 1979; Vijayalakshmi & Goswami , 1987; Goswami & Vijayalakshmi, 1990; Gotke *et al.*, 1990; Sangwan *et al.*, 1990; Akhtar & Mahmood, 1995; Abd-Aziz *et al.*, 1996; Alica & Sivaprakasam, 1996; Haseeb & Butool, 1996; Khurma *et al.*, 1997; McPortland,1997; Nagesh *et al.*, 1997; Abbott *et al.*, 1998; Al-Shalaby and Ali, 2001 and Amin and Farag, 2004). The aim of the present study, is to evaluate the nematicidal potential of some essential plant oils and yeast extract in reducing the population and development of *Meloidogyne incognita*

and *Rotylenchulus reniformis* and improve the corresponding growth of tomato plants.

MATERIALS AND METHODS

In this experiment, four essential oils or plant extracts of four plants belonging to four families; clove tree, *Syzygium aromaticum* (Merill & Perry); black cumin, *Nigella sativa* L.; orange peels, *Citrus sinensis* L.(Osbeck.); and tobacco, *Nicotiana tobacum* L. (water extracts 2% nicotine) and yeast extract, *Saccharomyces cerevisiae* (Table 1) were investigated against *M. incognita* and *R. reniformis* infecting tomato. One ml of each essential plant oil dissolved in one ml of 95% ethanol per pot was added and incorporated by shaking to one Kg sandy-loam soil (1:1 v/v) in 15 cm-d. plastic pot. In a comparable set, eight pots were treated with ethanol only. Five grams of yeast were each incorporated in four replicates one week before nematode inoculation. Two weeks later, one-month old seedlings of tomato cv Balady were singly transplanted in treated pots. After two weeks, each pot was inoculated with 1 000 infective stage of either *R. reniformis* or *M. incognita*. Un-inoculated four replicate pots were served as plant control. There were four replicates for each treatment. Plastic pots were arranged in a completely randomized design in a greenhouse at 35±5C° and watered daily. Fifteen days after nematode inoculation, tomato plants were carefully uprooted and nematodes in roots were counted by staining roots in boiling lactic-acid fuchsin solution for 2-3 min., cleaned in 45% lactic acid for 24 hours and examined under stereomicroscope. Percentages of reduction of female as compared to untreated plants were calculated. Length and weight of shoots and roots were also recorded. Data were statistically analyzed using least significant difference (LSD).

Table 1: Main components of plant and yeast extracts.

Botanical name	Common name	Family	Plant part	Main components*
SSyzygium aromaticum	Clove	Myrtaceae	Fruits	60-90% eugenol, eugenyl acetate, caryophyllene.
NNigella sativa	Black Cumin	*Ranunculaceae*	Fruits	50-60%apethol, limonene, 18-20%fenchone, phellandrene, pinene, anisic acid, sledhydre, camphene
CCitrus sinensis	Orange	*Rutaceae*	Peel	Over 90% Limonene , bergapten, auraptenol, and acid
NNicotiana tabacum	Tobacco	Solanaceae	Foliage	Nicotine
fSSaccharomyces cerevisiae	Backer's Yeast			C_{47} H $_{83}$ O_{33} N_9 P_{12} Salts$_{45}$ according to Barry, 1988) + microelements like (Potassium and Magnesium)

*According to Lawless (1992)

RESULTS

The present data showed that plant and yeast extracts significantly and variably reduced female numbers of *M. incognita* and ranged from 29.9% (black cumin) and 88.9% (yeast extract). Yeast and tobacco extracts were the

most effective in reducing the percentage of *M. incognita* females (88.9% and 85.2%) respectively followed by orange peel oil (33.3%), black cumin (29.6%) and clove tree oil (20.4%) (Table 2 and Fig. 1).

Table 2. Efficacy of some essential plant oils as well as tobacco and yeast extract on the final population of *Meloidogyne incognita* on tomato.

Treatment	Family name	Nematode final population in root/ plant					Female Reduc. %
		No. of galls	Devel opme ntal stages	No. of females	No. egg-laying females	Total no. of nemas	
Syzygium aromaticum	*Myrtaceae*	44.0	5.0	43.0	36.0	48.0	20.4
Nigella sativa	*Ranunculaceae*	34.0	20.0	38.0	33.0	58.0	29.6
Citrus sinensis	*Rutaceae*	31.0	11.0	36.0	32.0	47.0	33.3
Nicotiana tabacum	*Solanaceae*	9.0	6.0	8.0	6.0	14.0	85.2
Yeast		4.0	4.0	6.0	5.0	10.0	88.9
Inoculated plants (Check)		53.0	20.0	54.0	53.0	74.0	0.0
LSD (P>0.05)		7.5	3.7	9.4	8.6	10.4	-
LSD (P>0.01)		10.4	5.1	12.9	11.9	14.3	-

Each value presented the mean of four replicates.

Data presented in table 3 indicated that, the percentage of *R. reniformis* females reduction was between 80.5% (black cumin) and 92.6% (tobacco) and was between 79.8% (black cumin) and 96.1% (tobacco extract) for egg-laying females reduction. Tobacco extract was the most effective in reducing the population of females and egg-laying females (92.6% and 96.1% respectively), followed by clove oil (90.5% and 89.2% respectively) and yeast (90.0% and 91.6%).

Table 3. Efficacy of some essential plant oils as well as tobacco and yeast extract on the final population of *Rotylenchulus reniformis* on tomato.

Treatment	Family name	Nematode final population in root/ plant		Female Reduction %	Egg-laying Reduction %
		No. of swollen females	No. of egg-laying Females		
Syzygium aromaticum	Myrtaceae	5.5	5.5	90.5	89.2
Nigella sativa	Ranunculaceae	11.3	10.3	80.5	79.8
Citrus sinensis	Rutaceae	8.3	4.5	89.1	91.2
Nicotiana tabacum	Solanaceae	4.3	2.0	92.6	96.1
Yeast		5.8	4.3	90.0	91.6
Inoculated plants (Check)		57.8	51.0	0.0	0.0
LSD (P>0.05)		8.4	6.4	-	-
LSD (P>0.01)		11.5	8.8	-	-

Each value presented the mean of four replicates.

The maximum reduction of *R. reniformis* infection and reproduction was significantly achieved by using either yeast or tobacco extracts. Essential oils of black cumin, clove and orange peels were less effective in reducing *M.*

Incognita numbers than did in reducing *R. reniformis* (tables 2 & 3 and Fig. 1 &2).

Tomato plants showed luxuriant vegetative growth, In which weight and length of shoots were greatly Increased In plants grown In soil treated with yeast or tobacco extracts (tables 4 and 5).

Table 4. Effect of some essential plant oils of certain aromatic plants as well as tobacco and yeast extract on growth of tomato infected by *Meloidogyne incognita*.

Treatment	Family name	Shoot		Root	
		Length (cm)	Weight (g)	Length (cm)	Weight (g)
Syzygium aromaticum	Myrtaceae	29.0	9.5	19.6	12.0
Nigella sativa	Ranunculaceae	31.0	11.1	20.3	15.0
Citrus sinenses	Rutaceae	31.0	10.0	20.9	12.3
Nicotiana tabacum	Solanaceae	31.0	1.8	21.3	9.4
Yeast		34.0	12.3	23.0	15.0
Inoculated plants (Check)		25.5	9.0	15.3	8.3
Plant free of nematode inoculation		32.0	12.8	21.5	15.5
LSD (P>0.05)		2.4	1.2	1.7	2.2
LSD (P>0.01)		3.2	1.7	2.2	2.9

Each value presented the mean of four replicates.

Table 5. Effect of some essential plant oils of certain aromatic plants as well as tobacco and yeast extract on the growth of tomato infected by *Rotylenchulus reniformis*.

Essential plant oils	Family name	Shoot		Root	
		Length (cm)	Weight (g)	Length (cm)	Weight (g)
Syzygium aromaticum	Myrtaceae	28.3	8.8	18.8	11.0
Nigella sativa	Ranunculaceae	29.8	10.5	19.6	11.5
Citrus sinensis	Rutaceae	31.0	9.1	20.0	11.5
Nicotiana tabacum	Solanaceae	29.5	11.3	20.5	9.8
Yeast		32.3	12.5	22.3	12.5
Inoculated plants (Check)		23.8	9.8	14.5	8.5
Plant free of nematode inoculation		32.0	12.8	21.5	15.5
LSD (P>0.05)		1.9	1.4	1.8	1.4
LSD (P>0.01)		2.6	1.9	2.1	1.9

Each value presented the mean of four replicates.

DISCUSSION

It is evident from the present results that yeast or tobacco extracts gave the greatest reduction in numbers of *M. incognita* and *R. reniformis*. Whereas, the essential oils black cumin, clove tree and orange peels were less effective in reducing *M. incognita* than on *R. reniformis* on tomato cv. Balady. This effect may be due to the differential toxicity of essential oil compounds released during decomposition as suggested by Mahmood & Saxsena (1992). Presumably, it has been due to their quick decomposition or

evaporation before nematode inoculation. In most cases, highly significant and greater reduction in nematode populations occurred in soil where plant extracts were added to the soil infested with *R. reniformis* than those used the soil infested with *M. incognita*. This may be due to possible difference in the nature of reproduction and parasitism on tomato plants and the effect of released compounds. It has been suggested that during decomposition or evaporation of the essential oils in soil, a certain compound toxic to nematodes be released (Vats *et al.*, 1996; Hussaini *et al.*, 1997), which are dispersed within the soil pore spaces where most of the noxious population of nematodes occurred e.g. Abd-Elgawad and Omer (1995) found that the main compound of the volatile oil of *Marjorana hortensis, Mentha longifolia, Mentha spicata* and *Thymus vulgaris* were terpinen-4-ol (41.6%), carvone (70.4%), carvone (58.1%) and P- cymene (40.5%), respectivelly). Suggesting the inhibitory effect of such essential oils on the nematode final population, Alam (1991) reported that each time when plants were watered, the soluble fractions of essential oils products released into pore and penetrated the root tissues to kill the nematodes, thus reducing the final population below the threshold level. Other investigator suggested that soluble plant extract are very effective in inhibiting egg-hatch and larval motility of nematodes (Onifade & Fawole 1996, Tabil & Walia 1996).

It may be some plant extracts have a repellent or nematicidal properties (McPortland, 1997). Moreover, the soil fertility improves and increases plant tolerance to nematode infection. Similar results on the effect of vegetable oils and essential oils of aromatic and medical plants on the population of nematodes have been reported (Sangwan *et al.*, 1985, Sangwan *et al.*, 1990, Ali & El-Hamawi, 1995). Growth improvement of tomato plants may be due to additive effect of nutrients produced (Alam *et al.*, 1980). Besides the roots of plants grown in unfavorable circumstances for nematode penetration and feeding, inducing certain degrees of resistance against nematode attack. As a result of a reduction of nematodes, plant growth and yields were improved with essential oils treatments (Akhtar & Mahmood 1996; Firoza & Maqbool 1996 and Vats *et al.*, 1996). This may be due to the nematode reduction and partly due to the fact that this additive have also serve as organic manure's (Siddiqui & Alam, 1988). Among the effect of yeast extracts, a high and significant reduction was recorded on population of *M. incognita* and *R. reniformis* and significant improvements was noticed on tomato plants. Contrary results were reported by Youssef and Soliman (1997). They were found that, yeast extract gave the least reduction in nematode galls and egg-masses of *M.Incognita*. It can concluded that some plant extracts or oils could be considered as a bio-agent that may be limit the nematodes population densities below the threshold level. However, it seemed to be a safe and cheap method of control.

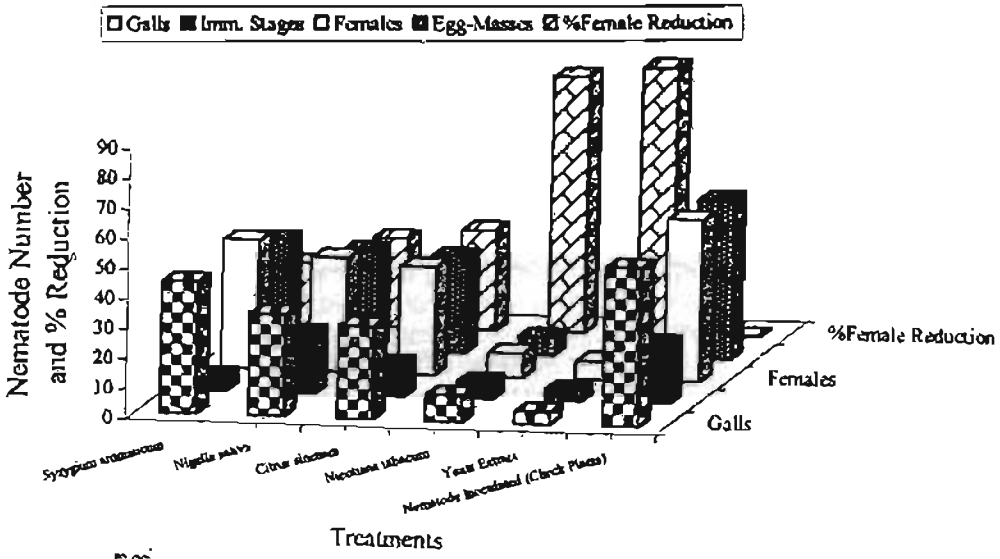

Fig. 1: Efficacy of some essential oils and yeast extract on number of galls, immature stages, females , egg-masses and the percentage of females reduction of *Meloidogyne incognita* on tomato.

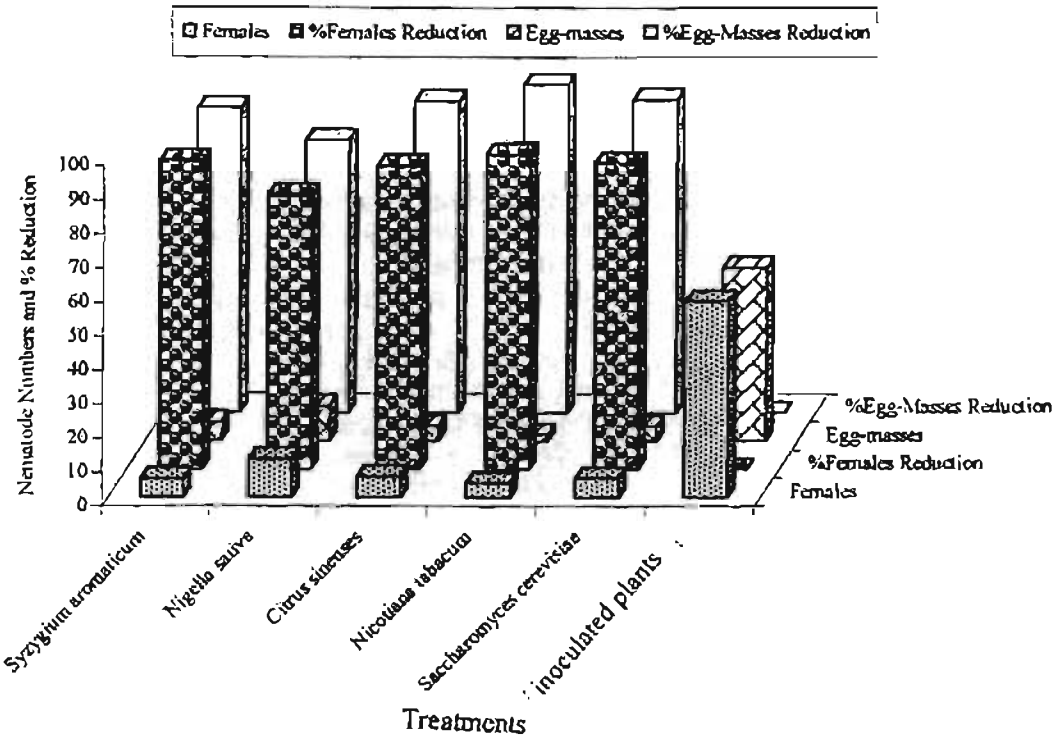

Fig. 2: Efficacyof some essential oils and yeast extract on females and egg -masses of *Rotylenchulus reniformis* on tomato.

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تقييم تأثير استخدام المستخلصات الزيتية النباتية لبعض النباتات و الخميرة في مكافحة نيماتودا تعقد الجذور (ميلودوجين انكوجنيتا) و النيماتودا الكلوية (روتيلنكيولس رينيفورمس) على الطماطم.

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تم تقييم أربعة من مستخلصات الزيوت النباتية و هى مستخلص زيت القرنفل و حبة البركة و مستخلص زيت قشر البرتقال و مستخلص نبات الدخان (نيكوتين ٢%) و التي تتبع العائلات: الاسية (Myrtaceae) و الشقيقية (Ranunculaceae) و السذبية (Rutaceae) و العائلة البازنجانية (Solanaceae) على التوالي مقارنة بمستخلص الخميرة (اضافه قطريه عضوية). و ذلك لدراسة قدرتها فى مكافحة كل من نيماتودا تعقد الجذور "ميلودوجين انكوجنيتا" و النيماتودا الكلوية "روتيلنكيولس رينيفورمس" فى الأصص البلاستيك مقاس ١٥ سم المملوء بواحد كيلو جرام من التربه الرملية الطميية بنسبه متساوية تحت ظروف البيوت الزراعية على نباتات الطماطم صنف بلدي.

تمت عدوى النباتات بألف من الأطوار المعدية بأي من نوعى النيماتودا المختبرة بعد اسبوعين من الإنبات فى تربة مخلوطة بواحد مل^{3} من المستخلص النباتي المذاب في كميه مساوية من الكحول الايثايل ٩٥% / اصيص و اضيفت الخميرة كمعاملة مستقلة بأذابه ٥ جرام من الخميرة الجافة فى ٥٠ مل من الماء و اضيفت للتربة قبل العدوى بأسبوع و تركت النباتات تنمو لمده ٦ أسابيع.

بعد حصاد التجربة (٦ أسابيع من العدوى) تم تسجيل أعداد العقد الجذرية و الأطوار الغير بالغة و أعداد الإناث و كتل البيض لنيماتودا تعقد الجذور و تسجيل أعداد الإناث و كتل البيض لنيماتودا الكلوية. و سجلت نسبة الانخفاض فى أعداد الإناث لكل من نوعى النيماتودا مقارنة بالكنترول (النباتات الغير معاملة و معداه بالنيماتودا). بالإضافة الى تسجيل أوزان و أطوال المجموع الخضري و الجذري.

و قد دلت النتائج على أن كل مستخلصات الزيوت المختبرة لها تأثير معنوي جدا فى خفض عدد العقد الجذرية و الأطوار المختلفة لنيماتودا تعقد الجذور (أطوار غير بالغة – أعداد الإناث و أعداد أكياس البيض) و أطوار النيماتودا الكلوية (أعداد الإناث و أعداد أكياس البيض) و بالتالي أدى الى مكافحة تلك الآفتين و أنعكس ذلك على زيادة فى النمو الخضري و الجذري فى معظم المعاملات. حيث انخفضت أعداد العقد الجذرية و الأطوار الغير بالغة و الإناث البالغة و أكياس البيض لنيماتودا تعقد الجذور بينما انخفض كل من أعداد الإناث و أكياس البيض المسجلة على الجذور للنيماتودا الكلوية و بالتالي انخفضت الأعداد الكلية للنيماتودا لكلا النوعين.

و قد لوحظ أن نيماتودا تعقد الجذور أقل تأثرا بمستخلصات تلك النباتات العطرية و كذلك مستخلص الخميرة حيث كانت نسبة التناقص فى أعداد إناث نيماتودا تعقد الجذور فى حالة المعاملة بمستخلص الخميرة (٨٨,٩%) و مستخلص نباتات الدخان (٨٥,٢%) و تلى ذلك زيت قشر البرتقال (٣٣,٣%) و زيت حبة البركة (٢٩,٦%) ثم زيت القرنفل (٢٠,٤). بينما كانت نسبه الانخفاض فى أعداد الإناث للنيماتودا الكلوية لمستخلص نباتات الدخان (٩٦,٢%) و لمستخلص الخميرة (٩١,٦%) يلى ذلك زيت قشر البرتقال (٩١,٢%) ثم زيت القرنفل (٨٩,٢%) و يليهم زيت حبة البركة (٧٩,٨%) و كذلك الأعداد الكلية للنيماتودا بالإضافة إلى العقد الجذرية و الأطوار الغير بالغة. و كان واضح أن كل من مستخلص الخميرة الجافة و مستخلص نباتات الدخان الاكثر تأثيرا على كل من نوعى النيماتودا.و قد لوحظ زيادة فى النمو الخضري والجذري نتيجة لانخفاض أعداد النيماتودا و زيادة فى معدل التسميد العضوي نتيجة تلك الإضافات. و هذه النتائج مشجعة للتطبيق بدون الدخول فى مشاكل التلوث بالكيماويات الزراعية.

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CONTROL OF THE EGYPTIAN COTTON LEAFWORM *Spodoptera littoralis* (BOISD.) BY USE OF FORMULATED BACTERIA.

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ABSTRACT

The efficacy of two pathogenic bacteria *Bacillus thuringiensis* var. *kurstaki* HD129 and *Serratia marcescens* were evaluated on 3rd and 5th larval instars of *Spodoptera littoralis*. These bacterial products were isolated by the Ain Shams Center of Genetic Engineering and Biotechnology.The commercial product *Bacillus thuringiensis* var. *kurstaki* "Protecto" was considered as a standard for comparison. Although, both two tested bacterial isolates had a high LC_{50} than the commercial product Protecto, both exhibited higher accumulative mortality. This effect was more apparent for *Serratia marcescens* than HD129 i.e. 66.5% and 62% respectively for treatment of 3rd instar. Furthermore, the time required to kill 50% of insects (LT_{50}) was the lowest when *Serratia marcescens* was tested, it was 4 days for both larval instars treated. Meanwhile, this period was 5 and 8 days for 3rd and 5th instar larvae treated with HD129 and it averaged 7 days for both treated instars when Protecto was used. Percentage of malformation was higher and number of larvae pupation was lower when the two tested bacterial isolates were tested than commercial product.

INTRODUCTION

Microbial control is alternatives to chemical control agents to insect pests and is often species specific. Microbial control agents although may not meet the speed of action of chemical insecticides. They have been generally shown to have no negative impacts on plants and mammals or even non-target insects . Bacterial biopesticides are dominated by *Bacillus thuringiensis* strains, meanwhile the virus nuclear polyhedrosis virus (known as NPV) generally plays a significant role.

The Egyptian cotton leaf worm *S. littoralis* is an insect pest of an economic importance with a wide range of host plants. This species has acquired resistance to many insecticides and the use of other control measures is essential to aid in an over all IPM. program Many lepidopteran species have been successfully controlled by microbial agents, e.g control of *S. littoralis* by *B.thuringiensis* [El-Hamaeky *et al.*, 1990; Salama *et al.*, 1993; Salem 1995;EL- Gahr *et al.*1995. ; Salama and Foda 1982; Salama *et al.* 1984] or control by using NPV (Salama *et al.* 1993; Harapaz and Wysoki 1984) .

The present study was conducted to evaluate the control effect of *B. thuringiensis* var. *kurstaki* HD129 and a strain of the bacterium *Serratia marcescens* on larvae of the cotton leaf worm *Spodoptera littoralis*.

MATERIAL AND METHODS

The original colony of the cotton leaf worm *S.littoralis* was obtained from a well-established culture, maintained at the Department of Plant Protection Faculty of Agriculture, Ain Shams University. Insect rearing was conducted in the laboratory as described by Youssef (1991).

Bacterial Cultures: -

The potency of two bacterial isolates were evaluated towards 3rd and 5th instar larvae of *S. littoralis*. The following isolates of bacteria were tested:

(I) *Bacillus thuringiensis* var. *kurstaki* [HD129]

(ii) *Serratia marcescens*.

These two isolates were kindly supplied as slants from Ain Shams Center of Genetic Engineering and Biotechnology to evaluate the efficiency of these two isolates, the commercial product *B. thuringiensis* var. *kurstaki* (Protecto) was used as a standard for comparison this product was obtained as a wettable powder from the Plant Protection Research Institute, Ministry of Agriculture, Cairo.

Maintenance of *B. thuringiensis* var. *kurstaki* (HD129): -

Subcultures from the bacteria *B. thuringiensis* var. *kurstaki* (HD129) were made by inoculation in a defined media of Pepton Yeast Extract as described by Mohammed (2002). The inoculated flasks were incubated at 30± 1^{0} C for 24h on a shaker set at 100-150 rpm. Pepton yeast extract agar plates were streaked by inoculate of the grown bacteria in the cultured test tubes using the streaking dilution method to obtain solitary pure colonies. Plates were incubated for 24h. at 30± 1^{0}C . Solitary colonies grown on the agar surfaces were selected and subcultured on agar slant and kept until needed for the experimental work.

Maintenance of the bacteria *S. marescens*: -

Subcultures from the bacterial samples *S. marcescens* were made by inoculation of Pepton Glycerol media. The inoculated flaskes were incubated at 30± 1^{0} C for24h on shaker (set 100-150 rpm.) to oblain solitary pure colonies Pepton Glycerol agar plates were streaked by inoculate of the grown bacteria in the cultured test tubes and incubated for 24h. at 30± 1^{0} C. Solitary colonies grown on agar surface were selected and subcultured on agar slants and reserved until required for the experimental work.

Bioassay for bacteria: -

One ml. of each of subculture HD129 and *Serratia marcescens* was placed in 100ml distilled water. As descriped by Schlegel (1986), series of dilutions were prepared [1%, .01%, 1x10^{-4}%, 1x10^{-6}%, 1x10^{-8}%, 1x10^{-10}%] from which the number of colony forming unit (cfu) were determined.

The commercial product Protecto (obtained as a wettable powder) series of dilutions were prepared from 1gm of the product [1%, .01%, 1x10^{-}

^{4}%, $1x10^{-6}$%, $1x10^{-8}$%, $1x10^{-10}$%]. Also the number of colony forming unit (cfu) were counted according to Schlegel (1986).

The larvicidal activity of the bacterial strains was evaluated on newly moulted 3rd and 5th instar of *S. littoralis* larvae. Fresh Castor oil leaves were cut in leaf discs, measuring 3 cm indiameter. These discs were immersed in each of the prepared dilution of each tested strain and then left to dry at room temperature before being offered to the 3rd and 5th instar larvae confined in plastic cups. Larvae well fed on contaminated leaf discs for 3 days and then provided with uncontaminated leaf discs for the subsequent duration of the larval instars. Each treatment was comprised 25 larvae and was replicated 5 times. The same numbers of larvae were considered as a control, which was offered castor oil leaves immersed in distilled water. Larval development as well as pupal survival and level of infection were considered, any malformation and sequence of infection were recorded. The sequences of symptoms of infection were recorded as well as larval development. Mortality was calculated daily and an accumulative larval mortality was determined at the end of the larval stage. Mortality percentages were corrected according to Abbott (1925) formula. Results were presented graphicelly as log/probit regression lines and LC_{50} values calculated by computer program Sigma plot for Windows (version 21). Furthermore, any malformation of larvae or pupa was recorded. As the standard commercial product Protecto is of known potency, the LC_{50} of the two tested bioagents HD129 and *Serratia marcescens*. Potency was calculated by the following formula, as described by Salama and Foda (1982).

$$\text{Potency sample (IU\mg)} = \frac{LC_{50}\ \text{standard}}{LC_{50}\ \text{sample}} \times \text{potency of standard (IU\mg).}$$

RESULTS

A range of concentrations was prepared from (HD129) and *S. marcescens*. These preparations were tested on 3rd and 5th instar larvae of *S. littoralis* (Boisd) HD129 and *S. marcescens*. toxicity was exhibited in a dose dependent phenomenon. Generally, the symptoms of the toxins to treated larvae could be summarized in the following sequence: -

(i) Loss of appetite as insect food consumption decreases as denoted by smaller castor oil leafs surface area eaten.
(ii) Decrease response to stimulation.
(iii) Diarrhea and larvae regurgitating vomiting of some fluids.

Furthermore, toxicity of HD129 was exhibited by the appearance of spots on the prolegs that then extend as dark brown on the abdomen then to the entire body. The larvae's body contents were soft to touch and the integument with a firm texture. Meanwhile, infection with *Serratia marcescens* toxins causes the appearance of a reddish pink pigmentation first on the prolegs that then extend on the whole integument, the infected larvae become very soft and the integument ruptures easily. Both tested bioagents lead to subsequent larval paralysis and death. From the plotted regression

lines the LC_{50} values of the tested toxins were determined, results are shown in Fig (1,2). Also LT_{50} and accumulative percentage mortality of larvae are exhibited in Table (1,2).

Table (1): Potency of at LC_{50} values on 3rd instar larvae of *Spodoptera littoralis*.

Bioagents	LC_{50} (cfu)	Slope	Potency (IU \ mg)	LT_{50} (days)	Accumulative % mortality (at the end of larval stage)
Protecto	40*105	0.37273	32000	7	56%
HD129	65*105	0.312715	52000	5	62.1%
S.marcesens	105*107	0.299380	84000	4	66.5%

(F) between treatments= 0.29327 (sign.) LSD=11.724

Table (2): Potency of at LC_{50} values on 5th instar larvae of *Spodoptera littoralis.*

Bioagents	LC_{50} (cfu)	Slope	Potency (IU \ mg)	LT_{50} (days)	Accumulative % mortality (at the end of larval stage)
Protecto	35*107	0.3171522	32000	7	62%
HD129	46*107	0.285920	42057	8	56%
S.marcesens	112*107	0.278338	102400	4	64%

(F)between treatments =1.32071 (sign.) LSD=8.310

Protecto, the commercials *B. thuringiensis* var. *kurstaki* was used as a standard in a range of concentration and LC_{50} determined under conditions of the present work (Fig (3)). It's LT50 and accumulative percentage are shown in Tables (1,2), it was obvious that this commercial product was the most toxic to *S. littoralis* larvae either treated as 3rd or 5th instars. The LC_{50} was $40X10^5$ and $35X10^7$ cfu. respectively HD129 was more toxic than *S. marcescens*. It's LC_{50} was $65X10^5$ and $46X10^7$ cfu for 3rd and 5th larval instars respectively. Meanwhile, for *S. marcescens* these values were $105X10^5$ and $112X10^7$ cfu for the respective mentioned instars. However, the LT_{50} of *S. marcescens* was slightly more rapid than HD129, as 50% of treated 3rd instar larvae died after 4 days approximately.

Meanwhile, LT_{50} was 5 days when HD129 was used, but was extended to 7 days when Protecto was used. As expected 5th instar larvae were much more tolerant than 3rdinstar's. This was evident for the two tested bioagents as well as the standard commercial Protecto. The accumulative percentage mortality (at the termination of the larval stage) was higher when *S. marcescens* was tested than for the use of HD129 or Protecto, (Tables 1,2). It was found to be 66.5 % and 64%for 3rd and 5th instars respectively as compared to 62 % and 56% when HD129 was tested for the respective mentioned larval instars.

The potency sample of *S. marcescens* was much higher than that of HD129, as this potency was 84000 and 102400 IU\mg for the treatment of 3rd and 5th instar larvae respectively, (Table 1,2). This value could be expressed a ratio increase of 1:16 and 1:24 than that of HD129. Some larvae of *S. littoralis* recovered from the toxins up on transfer to control diet.

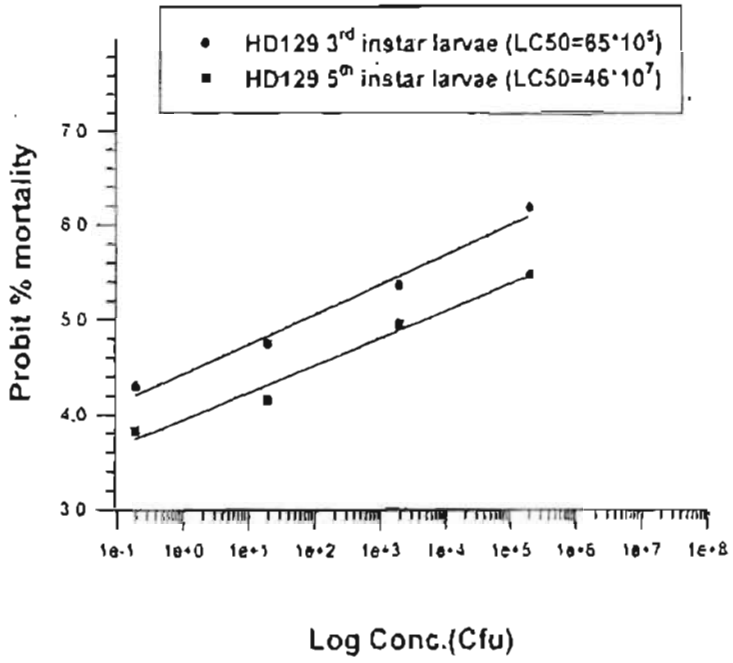

Fig (1) Effect of HD129 on 3rd &5th instar larvae of *S. littoralis*

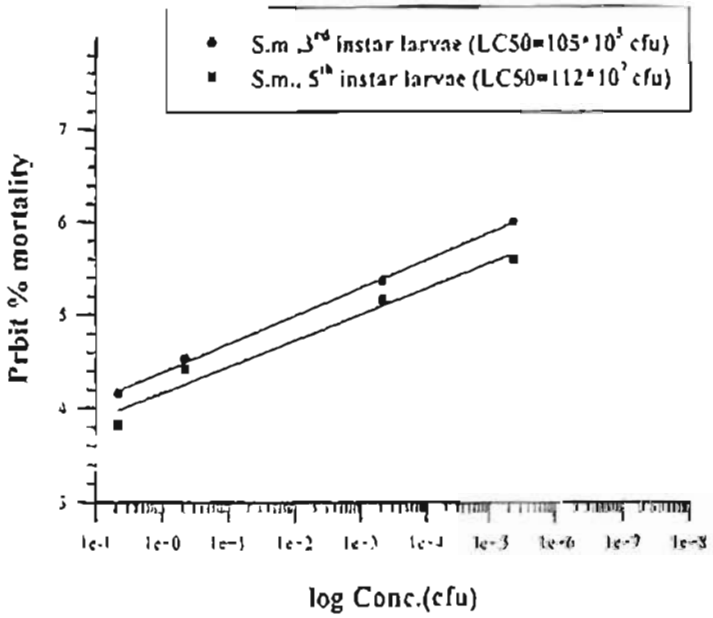

Fig (2) Effect of *S.marcescens* on 3rd &5th instar larvae of *S.littoralis*

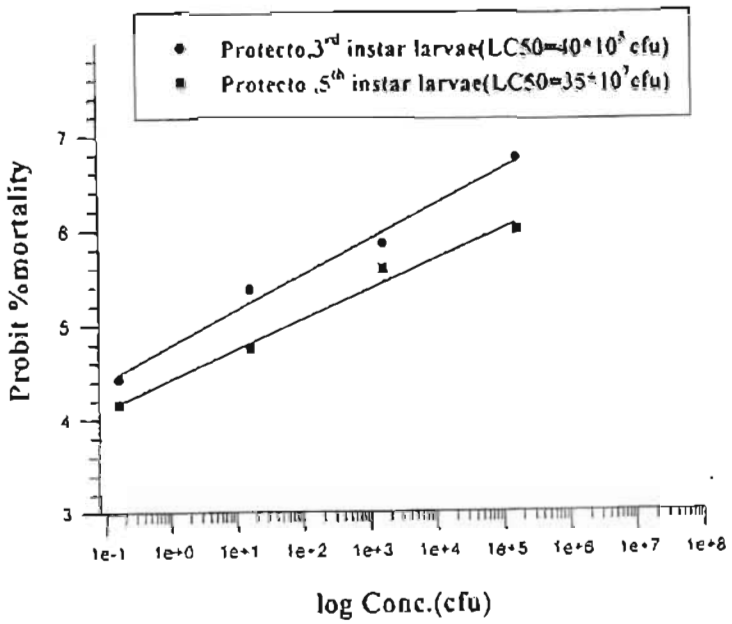

Fig (3) Effect of Protecto on 3rd &5th instar larvae of *S. littoralis*

Meanwhile, other larvae appeared with some abnormalities, which was more evident upon moulting (Fig 4). When 5th instar larvae were infected with LC_{50} of the tested HD129 and *S. marcescens* the duration of the subsequent instars of the larvae that survived was not significantly different than those of the control. Meanwhile, for the treatment of 3rd instar larvae, only the duration of the 6th instar was slightly impaired, the period of this last instar was shortened by 36-48 hours than the control. Following treatment with the two tested bioagents at LC_{50} value, the number of surviving larvae pupating was reduced between 30-36 % for both instars treated. Meanwhile, it was between 26-28 % when Protecto was used. The percentage of malformed pupae was more evident when 5th instar were treated, especially with treatment by LC_{50} of HD129 as it reached 12% as compared to 4 and 8 % when 3rd instar were fed on castor oil leaves contaminated with LC_{50} of *S. marcescens* and Protecto respectively.

Malformation of pupa was mainly observed as shortening of their length and appearance of larvae-pupal intermediates, (Fig 5), in all treatment adult ecdsion was totally inhibited as Insect failed to emerge as moths or died as pupa.

Fig (4): Malformed larvae of *Spodoptera littoralis* following treatment by LC_{50} of HD129 as 5th instar.

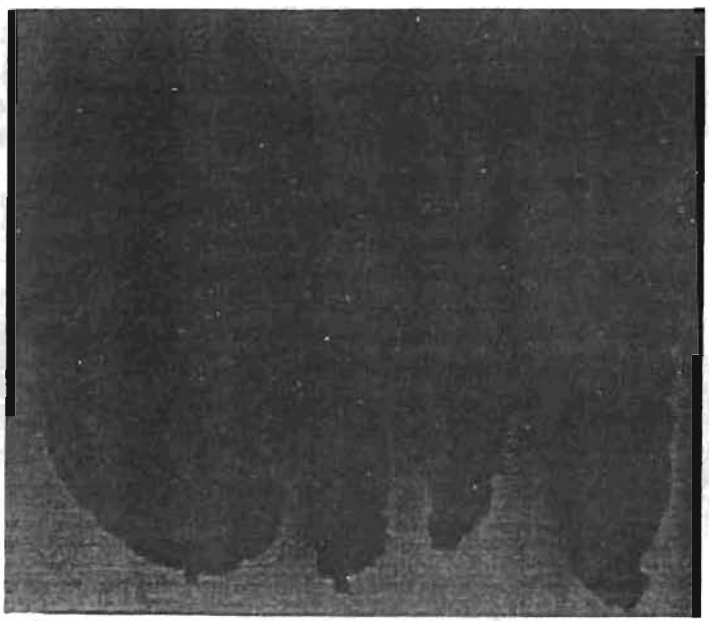

Fig (5): Malformed pupa of *Spodoptera littoralis* following treatment by LC_{50} of HD129 as 5th instar.

DISCUSSION

New isolates of bacteria have to be established so as to avoid the building of resistant of *S. littoralis* to this bacterial bioagents. In the present work a new isolates of *B. thuringiensis* var. *kurstaki* was tested i.e. (HD129) as well as the bacterium *S. marcescens*.

Potency of these two bioagents were compared with a standard commercial *B. thuringiensis* var *kurstaki* product Protecto widely used for the control of many lepidopterous insects [El- Hamaeky *et al.* 1990 , Vandenberg & Shimanuki 1990]. This commercial product proved to have a higher toxic effect than the other two bioagents investigated, as exhibited by it's much lower LC_{50} value. This is somehow expected as it being a commercial product it must has a longer persistence or activity as probable results of the addition of adjuvants or additives to achieve high efficacy. Meanwhile, HD129 and *S. marcescens* are newly prepared isolates. However the potency of the two tested bioagents was quite comparable, LC_{50} and potency sample of HD129 were much lower than that of *S. marcescens* which in contrast exhibited the highest LC_{50} value calculated. Meanwhile, Farrar *et al.* (1998) reported that the bacterial isolate *S. marcescens* killed the corn earworm with a very low oral dose. However, LT_{50} of *S. marcescens* was the lowest exhibiting 4 days for larvae treated either as 3rd or 5th instars. This period was the maximum-recorded i.e. 7 days when Protecto was used. It is a well-known fact that older larvae are usually much more tolerant to the toxic effect of many bioagents [Mohamed *et al.* 2000, Romeilah and Abdel-Megeed 2000].

This site of action of *B. thuringinese* toxin is the insect mid gut epithelium Gill *et al.* (1992) and one of the symptoms of poisoning is gut paralysis Gould and Anderson (1991). The bacterial bioagents do not cause a rapid kill, therefore their effect becomes apparent after a few days as infected insects eat little, later leading to starvation and death. Form the obtained results, it seems that *S. marcescens* caused much more rapid toxic effect, one or more factors are probably responsible for the potency of this toxin. Furthermore, the potency of *Serratia marcescens* when used at LC_{50} values was superior to Protecto and also slightly higher than HD129 to 3rd and 5th instars. This was exhibited in a higher accumulative mortality percentage as well as a higher reduction in larvae entering the pupal stage. Although, with higher LC_{50} value HD129 was more efficient for the control of *S. littoralis* larvae than Protecto. The binding characteristics of HD129 to the mid gut epithelium of the infected larvae could be involved as suggested by Herreo *et al.* (2001) and Gilliand *et al.* (2002).

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مكافحة دودة ورق القطن المصرية باستخدام مستحضرات بكتيرية

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١ - قسم وقاية النبات - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر.

٢ - قسم الوراثة - كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر.

تم فى هذه الدراسة تقييم اثنان من المستخلصات البكتيرية والتى تم استخلاصها بمركز الهندسة الوراثية كلية الزراعة جامعة عين شمس وهما بكتريا *Bacillus thuringiensis* var. *kurstaki* والمعروفة باسم (HD129) و بكتريا *Serratia marcescens* حيث اجرى التقييم على العمرين الثالث والخامس ليرقات دودة ورق القطن ، كما تم استخدام المركب التجارى البر وتكتو Protecto (*Bacillus thuringiensis* var. *kurstaki*) كمركب قياسى .وقد أوضحت الدراسة زيادة التركيز القاتل ل٥٠% من التعداد الكلى لليرقات لكلا المستخلصين عنه فى المركب القياسى مع زيادة نسبة الموت للتراكمية فى اليرقات المعاملة ببكتريا *Serratia marcescens* عنها فى اليرقات المعاملة ببكتريا (HD129) حيث وصلت الى ٦٦,٥% و ٦٢,١% للعمر الثالث لكلا المستخلصين على التوالى ، اضافة الى ان الفترة اللازمة لقتل ٥٠%من التعداد الكلى لليرقات كانت اقل عند استخدام بكتريا *Serratia marcescens* حيث كانت ٤ أيام لكل من العمرين الثالث والخامس مقارنة بـ ٨ أيام لكل من العمرين الثالث والخامس على التوالى عند استخدام بكتريا (HD129) أما عند استخدام المركب القياسى فكانت هذه الفترة ٧ايام ، كما أوضحت الدراسة أن النسبة المئوية للتشوه فى اليرقات والعذارى كانت اقل عند استخدام بكتريا (HD129) أو بكتريا *Serratia marcescens* عنها عند استخدام المركب القياسى.