Evaluate the performance of bioagents against *Tetranychus urticae in vitro*.

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Abstract: The spider mites *Tetranychus urticae* Koch and *Tetranychus evansi* Baker and Pritchard are important pests of horticultural crops. This study was conducted to determine the effect of some bioagentsa gainst *Tetranychus urticae*. Our results the data showed that the lowest median lethal concentration (LC_{50}) appeared non-significant on the mean number of *T. urticae* through different times Whereas, the high concentration of bioagents culture (100%) appeared significant increase inhibit the growth of *Tetranychus urticae* adult after 24 and 120 hours. that mortality percentage of *Tetranychus urticae* after 24, 48, 72. 96. 120 and 144 h respectively affected by 50 % and 100% culture of bioagents culture. Mortality ranged from 0 to 62 % during different time by 50 % culture of bioagents culture .*Beauveria bassiana and serratia* (Sm) recorded highly mortality percentage at different times. On the other hands, Mortality ranged from 0 to 55 % during different time by 50 % culture of bioagents culture. *Streptomyces sp* (STR) recorded highly mortality percentage at different times.
[ElSayed I. A. and Nada .O. Edrees. **Evaluate the performance of bioagents against *Tetranychus urticae in vitro*.** *J Am Sci* 2015;11(12):264-269]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 34. doi:10.7537/marsjas111215.34.

Key words: antagonistic - bioagents – biological control - *in vitro* – *Tetranychu surticae*

1. Introduction

Last fifty years the global population has grown more rapidly than ever before, questions arise concerning the capacity of current food production to support such growth (**Oerke and Dehne, 2004**). Agricultural products will be 60% higher in 2030 than present time and more than 85% of this additional demand will come from developing countries **(FAO, 2002)**. Many environmental factors effect on resources generated by agriculture. The damage caused by insects is one of the most important factors in the reduced productivity of crop plant species especially vegetables (**Oerke, 2006**).It is responsible for more or less 15% of today's crop losses.

Various vegetables are attacked by numerous insect pests, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most important pests that attacked the vegetables crops in the world (**Farouk & Osman 2009**). It is responsible for yielding losses to many horticultural ornamental and agronomic crops. The major problem in control of spider mites is destroyed of natural enemies by applications of insecticides against other pests (**Mainul Haque *et al.*, 2010**). Many spider mites have become resistant to most of the commonly used pesticides (**Van Lenteren and Woets, 1988**).

Nowadays, the hazards of synthetic pesticides on health and environmental are known. Pesticides are the primary method of control of *T. urticae*. However, there are problems associated with the use of pesticides for the control of this mite because populations develop resistance to acaricides and the chemicals leave residues on fruits **(Ahn *et al.*, 1998).**

Biological pest control is indispensable alternative to chemical pesticides. Apart from a reduction in production costs by using biological control to avoid environment pollution. Biological control has great potential for use against *T. urticae*as based on successes of biological control and due to the abundance of potential biological control agents. Several successful biocontrol agents have been developed and put into use over the years.

The aim of this study, was to evaluate the effect of microbial agent activity against the two spotted spider mite, *Tetranychus urticae* Koch *in vitro*.

2. Materials and methods

Bacterial strains and culture conditions:-

Bacterial strains (Table 1) were used in this study, which including their references, as well as, their designation. *Baterial* cells were cultivated in a Luria-Bertani (LB) medium (**Sambrook *et al.*, 1989**). The medium was supplemented with erythromycin at 10 mg/ml. The cells were routinely grown at 30 °C in 100 ml Erlenmeyer flasks with a culture volume of 20 ml in a rotary shaker at 200 rpm. The cultures were inoculated from overnight cultures (1%). Glucose solution was autoclaved separately and added to the culture medium before the inoculation to the final concentration of 1%.

T. harzianum strain and *Beauveria bassiana* grown on Potato Dextrose Agar (PDA) plates incubated at 25°C according to .**Senthamizhlselvan *et al.* (2010).**

Table 1. Bacterial strains used in this study.

Strains	Source or Reference	Designation
Serratiam arcescens	Agricultural Research Center (ARC)	Sm
Beauveria bassiana	Agricultural Research Center (ARC)	Bb
Trichoderma harzianum	Agricultural Research Center (ARC)	Tb
Pseudomonas fluorescences	Agricultural Research Center (ARC)	PF- 348
Bacillus thurinogensis	Bacillus Genetics stock Center, Biochemistry Dept., Ohio University. , Columbus, USA	4QSTR1
Streptomycessp	Agricultural Research Center (ARC)	STR

Stock culture of red spider mite

Adults of *Tetranych usurticae* were collected from the cucumber fields of. Leaves with spider mites were immediately transferred onto one-year old potted cucumber plants grown under green house conditions and used as stock culture. From the stock, adults were transferred onto fresh tea leaf squares (6 × 6 cm) placed on moistened cotton pads (0.5 inch thick) in plastic trays (42 × 30 × 6.5cm). Rearing trays were kept under controlled conditions of 25±1°C,75±5% RH and 16 L: 8 D photoperiod. Withered and dry leaves were regularly replaced.

Laboratory Bioassay

The tests were accomplished following **Campos *et al.* (1995).**To study the effect of biocontrol agents, against adults of red spider mite adopting on spraying of bacterial suspension at the concentrations of 50and 100% of the bioagents at time (24, 48, 72, 96, 120 and 142 h). The experimental arena consisted of 2 cm diameter cucumber leaf discs placed on moist cotton in Petri plates (9 cm diameter). Each treatment was replicated five times with ten mites per replication. The mites were introduced on the leaf discs and the suspension was sprayed from a distance of 25 to30 cm with a hand spray atomizer of 50 ml capacity until the leaf surface got just wet with very fine droplets. To eliminate mortality due to natural causes, this rate was corrected using the Abbott formula **(Abbott, 1925).**

Statistical analysis:

The obtained data of mite numbers were subjected to the analysis of variance test (ANOVA) with mean separation at 5% level of significance according to the method of **Snedecor and Cocharn (1967).**

3. Results and discussion

Research and development of biological control options for spider mites has largely concentrated on the conservation of natural enemies and releases of predatory mites (**Zhang, 2003**).The results of bioassays indicate that the differences in the susceptibility of adult *T. urticae* against sex bioagents. Results present in Tables 2and 3 showed that mean number of two spotted spider mite *Tetranychus urticae* spraying of six bioagents used in this study with two concentrations 50 and 100 % of bioagents culture. The data showed that the lowest median lethal concentration (LC50) appeared non-significant on the mean number of *T. urticae* through different times (Table 2). This results in agreed with **Ghosh *et al.* (2007)** who found that *B. bassiana*, more virulent against *T. urticae* adult. As shown from the results presented in Table 3, the highest concentration of bioagents culture (100%) appeared significant increase inhibit the growth of *Tetranychus urticae* adult after 24 and 120 hour. These results in agreed with **Xian *et al.*, (2001)who** reported that a *Verticillium lecanii* could bring down *T. urticae* population by 70-80% after 14 days of spraying on greenhouse vegetable crops. In a specific case**, Vodovar *et al.* (2006)** reported that The cells of *P.fluorescens* were introduced on to the body surface of mites either indirectly by brushing their ventral surface against the surface of leaf disc or by cleaning their *mouthparts*. Whereas, **Pena *et al.* (1996)** found that fungal isolates originating from *Polyphagotarsonemus latus* Banks (Tarsonomidae) were more pathogenic to this mite species than those isolated from other hosts. Although strict adaptation of strains of *M. anisopliae* to the original host has been reported in case of scarabaeid beetles **(Ferron *et al.*, 1972)**, *M. anisopliae* and *B. bassiana*are ubiquitous pathogens recorded on many hosts (**Veen 1968**). The lethal concentration values obtained in this study were in the range of those reported by other workers on mite hosts such as *Tetranychus urticae* Koch and *V. destructor* **(Shaw *et al.*, 2002). Oliveira *et al.* (2007**) found that *P. macropilis* can be an efficient biocontrol agent of spider mites.

The results summarized in diagrammed in Figures 1:6 showed that mortality percentage of *Tetranychu surticae* after 24, 48, 72. 96. 120 and 144 h respectively affected by 50 % and 100% culture of bioagents culture. Mortality ranged from 0 to 62 % during different time by 50 % culture of bioagents culture. *Beauveria bassiana and serratia* (Sm) recorded highly mortality percentage at different times. On the other hands, Mortality ranged from 0 to 55 % during different time by 50 % culture of bioagents culture .*Streptomyces sp*(STR) recorded highly mortality percentage at different times. This results agreed with **Tamai *et al.* (2002)** screened 45

isolates of mitosporic fungi against *T. urticae*, including 32 isolates of *Beauveria bassiana* (Balsamo) Vuillemin, 10 isolates of *Metarhizium anisopliae* (Metchniko V), one each of *Aschersonia aleyrodis* Webber, Hirsutella sp. And Isariafarinosa (= *Paecilomyces farinosus*) (Holmsk.) Fr. Among these isolates, eight *B. bassiana* and four *M. anisopliae* isolates caused >80% and 90% mortality, respectively,at concentration of 5 £ 107 conidia ml, 5 days post infection. In a study by **Tamai (1998**) on the mite *T. urticae*using *Beauveri a* spp. isolates at a concentration of 5 × 108 conidia ml-1 mortality ranged from 5.50% to 100% were observed. Similarly, de **Oliveira *et al*. (2002),** worked with *B. bassiana* isolates at 108 conidia ml-1 and the red mite *Oligonychu syothersi* (McGregor), recorded 77.00% to 98.00% mortality. On the other hand isolates of *M. anisopliae*, caused 12.00% to 45.00%, and LT50 values that ranged from 8.6 to 18.4 days.

Table 2. Mean number of *Tetranychus urticae*/ leaf under laboratory conditions affected by 50 % culture of bioagents culture.

Bioagents	24h	48h	72h	96h	120h	142h
Control	12	13	15	13	11	15
Tb	9	8	8	12	7	8
Bb	8	8	10	10	9	10
PF- 348	9	10	10	10	11	9
STR	10	10	10	10	9	9
4QSTR1	11	12	13	11	10	11
Sm	9	5	5	6	6	6
F-test	NS	NS	NS	NS	NS	NS

Table 3. Mean number of *Tetranychus urticae*/ leaf under laboratory conditions affected by100 % culture of bioagents culture.

Bioagents	24h	48h	72h	96h	120h	142h
Control	10	13	14	11	10	10
Tb	7	6	7	7	6	6
Bb	5	6	8	9	6	6
PF- 348	7	9	9	11	10	10
STR	10	10	10	9	9	9
4QSTR1	9	10	10	10	8	9
Sm	9	5	3	3	3	3
F-test	*	NS	NS	NS	*	NS
LSD 5%	2.14				4.81	

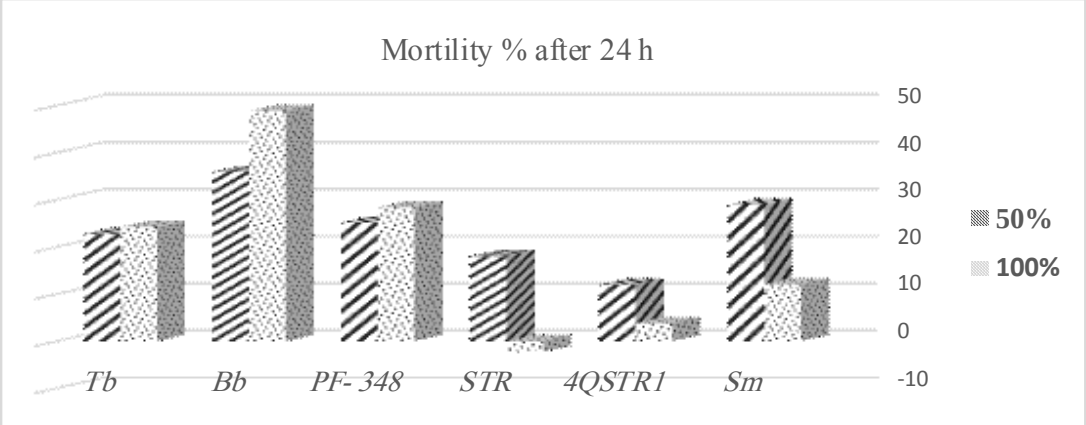

Fig 1. Mortality percentage of *Tetranychus urticae* after 24 h affected by 50 % and 100% culture of bioagents culture.

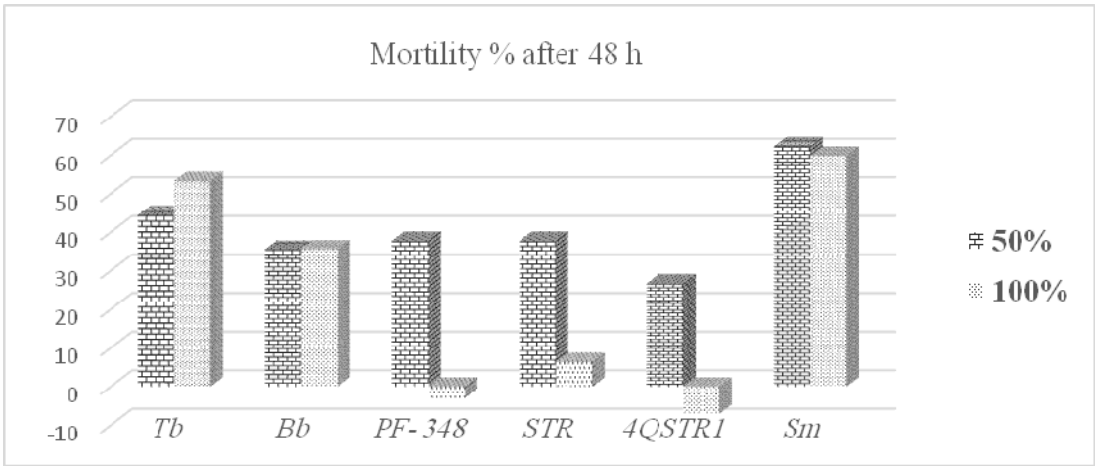

Fig 2. Mortality percentage of *Tetranychus urticae* after 48 h affected by 50 % and 100% culture of bioagents culture.

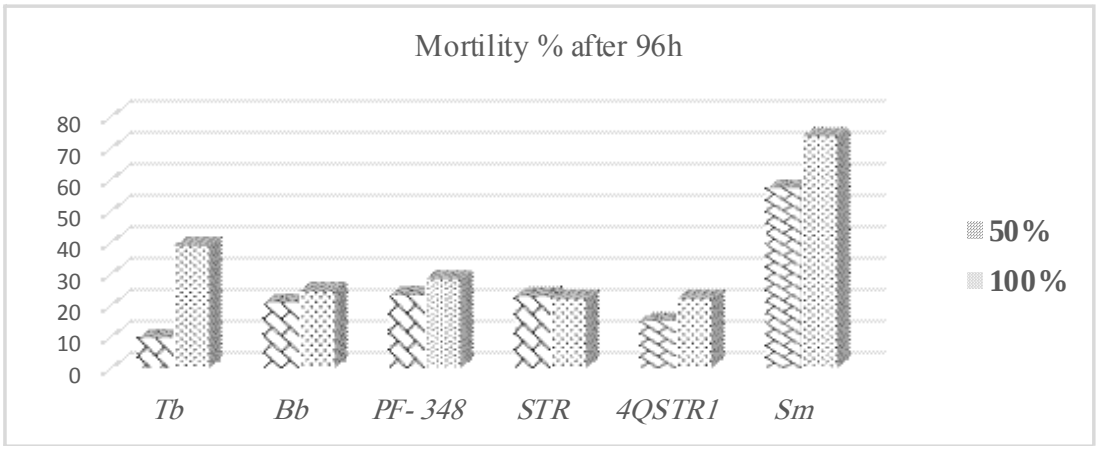

Fig 3. Mortality percentage of *Tetranychus urticae* after 96 h affected by 50 % and 100% culture of bioagents culture.

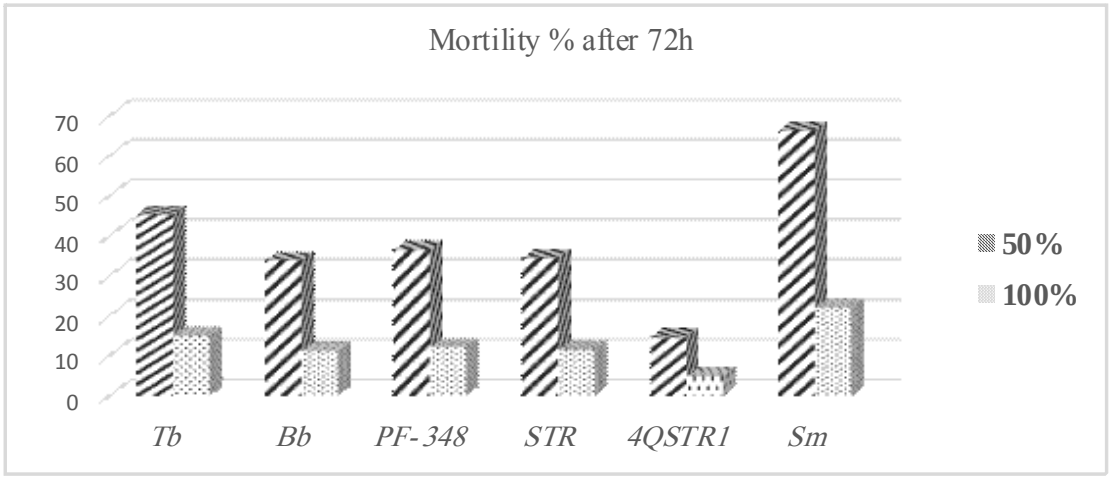

Fig 4. Mortality percentage of *Tetranychus urticae* after 72 h affected by 50 % and 100% culture of bioagents culture.

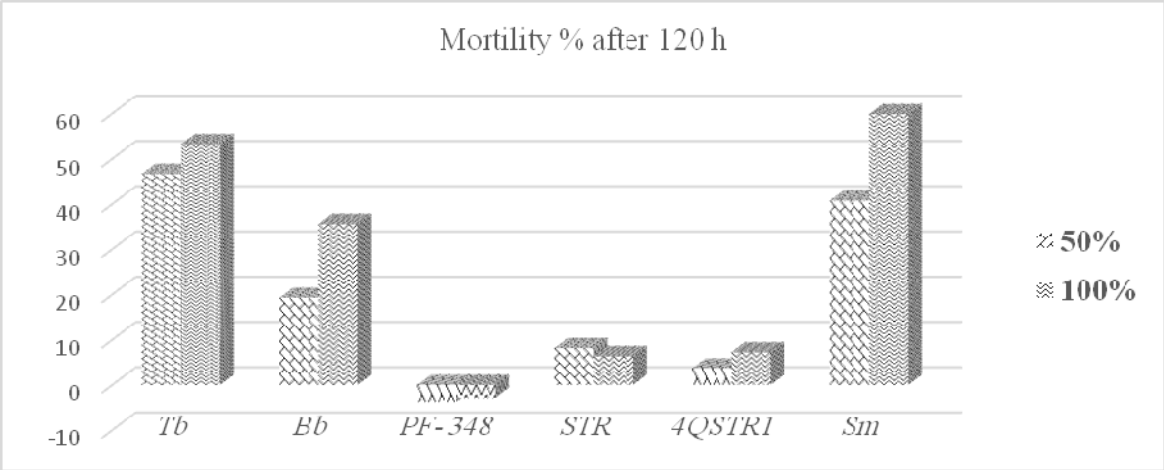

Fig 5. Mortality percentage of *Tetranychus urticae* after 120 h affected by 50 % and 100% culture of bioagents culture.

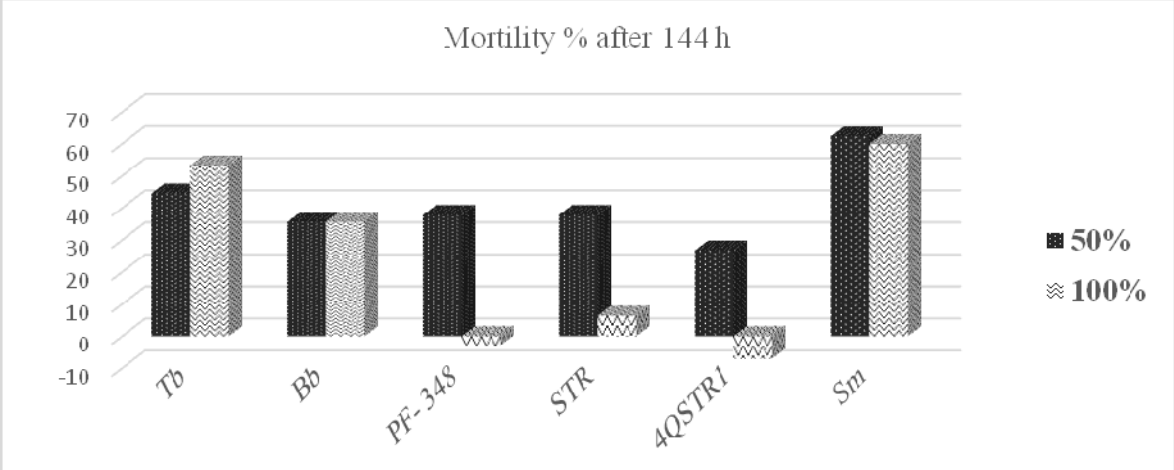

Fig 6. Mortality percentage of *Tetranychu surticae* after 144 h affected by 50 % and 100% culture of bioagents culture.

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12/25/2015