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EFFICIENCY OF *Trichoderma harzianum* AND SOME ORGANIC ACIDS ON THE COTTON BOLLWORMS, *Earias insulana* AND *Pectinophora gossypiella*

El – Massry, S. A.A.; Hend Gh. Shokry and M. E. M. Hegab
Plant Protection Research Institute, Dokki, Giza, Egypt

ABSTRACT

Larvae of spiny bollworm, *Earias insulana* and pink bollworm, *Pectinophora gossypiella* treated with *Trichoderma harzianum* liquid filtrate and spores of the fungus was investigated. In addition, the toxic effect of salicylic and tannic acids on the two insect species was studied. Bioassays were performed using a fungal filtrate 1, 0.5 and 0.25 ml (V/V) and spores suspension concentrations (2×10^5, 1×10^5 and 0.5×10^5 spores / ml). The results showed that the mortalities of *E. insulana* and *P. gossypiella* larvae were closely related to the rates of the filtrate and the spores of *T. harzianum*. After infection with the fungal filtrate for 3 days, the three tested rates recorded the same mortality 60% of *E. insulana*. But, the mortality of *P. gossypiella* was 40, 33.33 and 31.67% for 1, 0.5 and 0.25 ml, respectively. On the other hand, after the same period of infection the concentrations of *T. harzianum* spores 2×10^5, 1×10^5 and 0.5×10^5 spores / ml gave 60, 53.33 and 50% mortality and 80, 76 and 75% mortality for *E. insulana* and *P. gossypiella*, respectively. The toxic effect of salicylic acid on *P. gossypiella* was higher than its toxicity against *E. insulana*. After 13 days of treatment, it was exhibited 50, 48, 46, 45 and 45% mortality of *E. insulana* and 86.67, 83, 80, 78 and 76.67% mortality of *P. gossypiella* at the concentrations 1900, 1425, 950, 475 and 237.5 ppm, respectively. Tannic acid gave 70, 65, 63, 62 and 62% mortality for *E. insulana* and 45, 42, 40, 36.66 and 31.66% for *P. gossypiella* at the tested concentrations 2000, 1500, 1000, 500 and 250 ppm, respectively.

Keywords: *Earias insulana*, *Pectinophora gossypiella*, *Trichoderma harzianum*, salicylic acid, tannic acid.

NTRODUCTION

In Egypt, during the recent years, cotton plants suffer from the infestation with spiny bollworm, *Earias insulana* and the pink bollworm, *Pectinophora gossypiella*. The loss caused by *P. gossypiella* to cotton arises to one million kentar annually (Metwally *et al*., 1980). There is a serious interest in the use of microbial insecticides for biological control of insect pests as alternatives to chemical control, since they leave toxic chemical residues in the environment and induce resistance in their insect hosts (Evans, 1999). Entomopathogenic fungi in common with other insect natural enemies can be employed for biocontrol strategies (Shah and Pell 2003). *Trichoderma harzianum* have been used for biocontrol of the different pests of crop plants in addition to it's ability to produce some effective antimicrobial agents for controlling plant diseases. The spore suspension and metabolites of this fungus showed a high pathogenic effect on the Egyptian cotton leafworm, *Spodoptera littoralis* larvae (Ashraf and Momein, 2007). The organic acids also considered a new effective mean for control the agriculture pests and it is safely used due to it's quickly degradation in the soil (Shokry, 2013). The salicylic acid pathway plays an important role in the protection of plants against the herbivorous insect. The pathways also interact with salicylic acid having an inhibitory effect on the octadecanoid pathway and vice versa. This acid also reported to act synergistically (Remco *et al*., 2002). In the same trend, tannic acid have also an adverse effect on the different insects by reducing their digestive efficiency and growth (Manuwoto and Scriber, 1986).

The purpose of this study is to explore the pathogenic effect of the liquid culture filtrate and spore suspension of the fungus *T. harzianum* on the 1st instar larvae of *E. insulana* and *P. gossypiella* bollworms under laboratory conditions. The toxic effect of salicylic acid and tannic acid on these larvae species was also investigated.

MATERIALS AND METHODS

Experimental insects:

Newly hatched larvae (two days old) of *Earias insulana* and *Pectinophora gossypiella* were reared on artificial diet culture in the Bollworm Research Department, Plant Protection Research Institute (Sharkia branch). The two larvae species were reared in the incubator at 26 ±1°C and 80 ± 5 % R.H. *E. insulana* reared on artificial diet described previously by Amira, and Ammar (1985). But the larvae of *P. gossypiella* were reared in culture described by Abd El-Hafez *et al*. (1982).

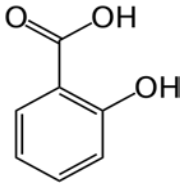
Entomopathogenic fungus:

A *Trichoderma harzianum* strain was obtained from the Insect Pathogen Unit (IPU), Plant Protection Research Institute, Agricultural Research Center, Egypt. Before the experiment, the strain was cultured on potato dextrose agar medium (PDA) for 15 days at 25 ± 1°C, with 75 ± 10% RH (Jinhua *et al*., 2013).

Tested organic acids:

A- Salicylic acid

- Trade name: Mediplast (95% powder) white crystalline powder.
- Structure formula:

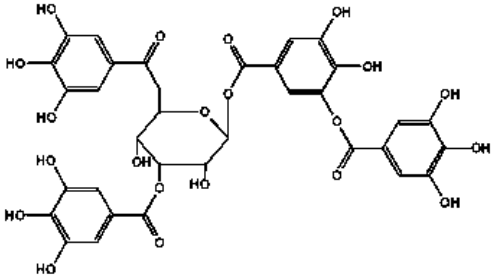


- Chemical name: 2 – Hydroxy – benzoic acid.

B- Tannic acid

- Trade name: Tannic acid (100% powder) brown powder.

- **Structure formula:**



-Chemical-name:2,3-dihydroxy-5{[(2R,3R,4S,5R,6R)-3, 4, 5, 6- tetrakis ({3, 4- dihydroxy-5 - [(3,4,5-trihydroxyphenyl) carbonyloxy]phenyl}carbonyloxy)oxan-2-yl]methoxy}carbonyl)phenyl3,4,5-**trihydroxybenzoate**

* These acids were obtained from El – Gomhouria Company, Egypt.

Fungal suspension preparation:

After culture for 15 days in agar slants, the fungal spores were harvested by rinsing in 10 ml sterilized distilled water containing 0.01% Tween-80, then filtered through cheese cloth. The spore concentration was determined using a hemocytometer and adjusted to 2×10^5, 1×10^5 and 0.5×10^5 spores / ml (Shokry, 2007).

Fungal filtrate preparation:

The tested fungus was inoculated into 50 ml liquid sterilized PDA in glass bottle (100 ml). The bottle incubated at 25°C for 10 days. After culturing, the cultured broth was filtrate by using filter paper. The filtrate rates which prepared were 1, 0.5 and 0.25 ml.

Acid concentrations preparation:

Five concentrations of 1900, 1425, 950, 475 and 237.5 ppm were prepared from salicylic acid and 2000, 1500,1000, 500 and 250 ppm from tannic acid. These concentrations prepared by solving the amount of each tested acid required to obtain the appropriate concentration in distilled water (Mahmoud, 1994).

Bioassays:

Five grams of kidney been artificial diet were put in a Petri dish (7.5 × 2 cm). One ml from each tested rate of the fungal liquid filtrate or fungal spore suspension was added to the surface of the diet, and then left until dryness. Control plates without fungal infection were prepared. Twenty five newly hatched larvae of *E. insulana* and *P. gossypiella* were transferred to treated artificial diet in each plate then left to feed. Petri dishes were covered by fine and soft paper below the glass cover and placed in an incubator at 26 ±1ºC and 80 ±5% R.H. Three replicates for every treatment and control were done. The mortality percentages were recorded for each plate daily for 15 days (Zaki, 2014).

Toxicity of acids:

One ml from each experimental concentration of each salicylic acid and tannic acid was added to the surface of the diet which introduced into petri –dishes. Control plates prepared without any chemical treatment. Twenty five newly hatched larvae of *E. insulana* and *P. gossypiella* were put in each plate containing treated artificial diet and in the control plates. Petri dishes were covered by fine and soft paper below the glass cover and placed in an incubator adjusted at 26 ±1ºC and 80 ±5% R.H. Each tested concentration of each acid and control were replicated three times. The mortality percentages were calculated for all plates daily for 13 days.

Statistical analysis:

The obtained data in each control method were statistically analyzed and the treatment means were compared according to the method of CoStat (2005) statistical program analysis, computer program software.

RESULTS AND DISCUSSION

Effect of the fungal filtrates on *E. insulana* and *P. gossypiella* larvae:

The results presented in Table (1) reveals that *E. insulana* highly affected by *T. harzianum* filtrate than *P. gossypiella* species. 60% was the mortality which recorded by the all tested rates against *E. insulana*. While, 40, 33.33 and 31.67% were the mortalities which exhibited by 1, 0.5 and 0.25 ml against *P. gossypiella*, respectively. These mortality percentages were recorded after 3 days of infection and still stable till the end of experiment (15 days). On the other hand, obtained data showed a highly significant difference between the all tested concentrations comparing with the untreated one in case of the two larvae species.

These results are supported by Binod *et al*. (2007) reported that the culture filtrate of *T. harzianum* is capable of negatively affecting the growth and metamorphosis of *Helicoverpa armigera* larvae. It is also a potent antifeedant as it reduced the feeding rate and body weight of the larva. On the other hand, this fungus filtrate reduced the successful pupation and caused 70% mortality of this larva. Vijaykumar *et al*. (2009) evaluated the effect of *T. harzianum* culture filtrate against the major cotton pests (*Helicoverpa*, *Earias* and *Pectinophora* spp.). Results stated that *T. harzianum* culture filtrate showed the highest mortality for the all tested pest species at 2000 U / ml. The effect of *Streptomyces* culture filtrate on the 1st instars of cotton leafworm *Spodoptera littoralis* was less than the pellets of this genus (Osman *et al*., 2007). *Trichoderma viride* filtrate caused mortality of the pupa and larvae of silkworm, *Bombyx mori* (Berini *et al*., 2015).

Effect of the fungal spores on *E. insulana* and *P. gossypiella* larvae:

As shown in Table (2) *P. gossypiella* was more sensitive to *T. harzianum* mould spores than *E. insulana*. After three days, 2×10^5, 1×10^5 and 0.5×10^5 spores / ml gave extensive mortality reached to 80, 76 and 75% of *P. gossypiella* larvae, respectively. But the same concentrations recorded 60, 53.33 and 50% mortality in case of *E. insulana* larvae, respectively. These mortality percentages still stable till the end of experiment. The illustrated data indicated also a highly significant difference between the all tested concentrations comparing with the untreated one for the larvae of the two species.

Table (1): Effect of *T. harzianum* filtrate on the 1st instar larvae of *E. insulana* and *P. gossypiella* at laboratory conditions

Rates (ml)	Mortality %	
	E. insulana	*P. gossypiella*
1	60.00[a]	40.00[a]
0.5	60.00[a]	33.33[b]
0.25	60.00[a]	31.67[b]
Control	5[b]	3.33[c]
F. Test	***	***
L.S.D. $_{0.05}$	14.12	6.66

The same letter in the same column means not significant at P < 0.05

Table (2): Effect of *T. harzianum* spores on the 1st instar larvae of *E. insulana* and *P. gossypiella* at laboratory conditions

Concentrations (Spores/ml)	Mortality %	
	E. insulana	*P. gossypiella*
2×10^5	60.00[a]	80[a]
1×10^5	53.33[ab]	76[a]
0.5×10^5	50.00[b]	75[a]
Control	5.00[c]	3.33
F. Test	***	**
L.S.D.$_{0.05}$	8.59	14.38

The same letter in the same column means not significant at P < 0.05

These results are in direct contradiction of the reports by Jassim *et al*. (1990) who cleared that *T. harzianum* has a larvicidal activity on the elm bark beetle *Scolytus* spp. It has also high activity on the larvae of the mealworm *Tenebrio obscurus* (Shakeri and Foster, 2007). The aqueous spore suspension of *T. harzianum* recorded 80% mortality of *S. littoralis* larvae when applied at 1×10^8 spore m^{-1}. This larvae also showed immune – dependant sensitivity to the fungus *Beauveria bassiana* while, *Aspergillus flavus* not has any effect against the larvae species (Ashraf and Momein, 2007). On the other hand, Hegab and Zaki (2012) reported that biovar (*Beauveria bassiana*) at concentration 32×10^5 spores / mg achieved 15.55% mortality of the spiny bollworm, *Earias insulana* larvae after six days of infection. At the same trend, Jinhua *et al*. (2013) showed that the conidial suspension of the fungus *Beauveria brongniartii* has a high pathogenic effect against the larvae of *Dendrolimus tabulaeformis*.

Toxic effect of organic acids on the larvae of *E. insulana* and *P. gossypiella*:

Results in Table (3) cleared that after one day of treatment, salicylic acid recorded 36.67, 25, 18.33, 16.66 and 11.66% mortality of *E. insulana* larvae at 1900, 1425, 950, 475 and 237.5 ppm concentrations, respectively. While, the same concentrations gave 36.66, 28.33, 23.33, 18.33 and 18.33% mortality of *P. gossypiella* larvae, respectively. The mortality of the two larvae species increased gradually by increasing the experiment period. After 9 days of treatment, the mortality reached to 50, 48, 46, 45 and 45% for *E. insulana* larvae at the concentrations 1900, 1425, 950, 475 and 237.5 ppm, respectively. But after the same period, the same concentrations gave 86.67, 81.67, 80, 78 and 76.67% mortality of *P. gossypiella* larvae, respectively. The mortality percent of the two larvae species still stable till the end of experiment except in case of the concentration 1425 ppm which increased the mortality of *P. gossypiella* larvae to 83% after 13 days of experiment. Obtained data showed also a high significant difference between the all concentrations of salicylic acid comparing with the control for larvae of the two species. These results are agree with those obtained by War *et al*. (2015) cited that salicylic acid able to reduce the weights and survival of *Helicoverpa armigera* larvae, suggesting that this acid can be used as a component of pest management in different plants. The effect of salicylic acid on insects would vary among plant and insect species (Heil and Bostock, 2002). The pathway of this acid plays an important role in the protection of plants against many pathogen species (Dempsey *et al*., 1999). At the same direction, Abdul-Rashid *et al*. (2012) stated that salicylic acid killed insects by damage the digestive system of these insects. Salicylic acid showed 67% mortality of bollworm *H. armigera* at the concentration 1.0mM after 24 h. of treatment. While, 100% mortality was observed after 96 h. with only two concentrations of salicylic acid 1.0 and 1.5Mm. On the other hand, salicylic acid recorded 51% mortality of the spotted bollworms, *Earias vitella* (Nighat *et al*., 2008). Hussein *et al*. (2014) reported that salicylic acid reduced 37.5% of *Tuta absoluta* at dose equal 200 mg / L and ascorbic acid at 200 ppm dose can also reduced *T. absoluta* damage.

Table (3): Toxic effect of salicylic acid on the 1st instar larvae of *E. insulana* and *P. gossypiella* at laboratory conditions

Insect species	Conc. (ppm)	Mortality % at indicated days 1	3	6	9	13
E. insulana	1900	36.67^{a}	41.67^{a}	43.33^{a}	50.00^{a}	50.00^{a}
	1425	25.00^{b}	33.33^{b}	41.66^{a}	48.00^{a}	48.00^{a}
	950	18.33^{c}	32.00^{b}	40.00^{a}	46.00^{a}	46.00^{a}
	475	16.66^{cd}	31.66^{b}	40.00^{a}	45.00^{a}	45.00^{a}
	237.5	11.66^{d}	30.00^{b}	40.00^{a}	45.00^{a}	45.00^{a}
	Control	5.00^{e}	5.00^{c}	10.00^{b}	10.00^{b}	10.00^{b}
	F. test	**	**	**	**	**
	L.S.D.$_{0.05}$	6.13	5.46	5.33	7.66	6.62
P. gossypiella	1900	36.66^{a}	68.33^{a}	80.00^{a}	86.67^{a}	86.67^{a}
	1425	28.33^{b}	65.00^{ab}	75.00^{ab}	81.67^{ab}	83.00^{ab}
	950	23.33^{c}	62.00^{b}	73.00^{b}	80.00^{b}	80.00^{bc}
	475	18.33^{d}	60.00^{bc}	70.00^{b}	78.00^{b}	78.00^{c}
	237.5	18.33^{d}	55.00^{c}	70.00^{b}	76.67^{b}	76.67^{c}
	Control	8.33^{e}	10.00^{d}	10.00^{c}	10.00^{c}	10.00^{d}
	F. test	**	**	**	**	**
	L.S.D.$_{0.05}$	4.65	8.51	6.25	6.35	5.90

The same letter in the same column means not significant at P < 0.05

As indicated in Table (4) *E. insulana* larvae highly affected by tannic acid than *P. gossypiella*. After one day of treatment, tannic acid recorded 51.67, 43.67, 41.67, 30 and 28.33% mortality of *E. insulana* at 2000, 1500, 1000, 500 and 250 ppm concentrations, respectively. While, after the same period the same concentrations exhibited 26.66, 21.66, 18.33, 15 and 8.33% mortality of *P. gossypiella*, respectively. As regarding, the mortality of the two larvae species increased by increasing the tested concentrations and the experiment period. After 13 days of experiment, the mortality of *E. insulana* reached to 70, 65, 63, 62 and 62% and 45, 42, 40, 36.66 and 31.66% for *P. gossypiella* at the concentrations 2000, 1500, 1000, 500 and 250 ppm, respectively. The differences in the larval mortality of the two larvae species were highly significant in comparing with the control. These results are in harmony with those reported by Kubo *et al.* (2003) stated that the larvae of *Pectinophora gossypiella* are sensitive to tannic acid. The same acid acts as a toxin against *Malacosoma disstria* and *Orgyia leucostigma* larvae (David, 1989). On the other hand, tannic acid caused mortality of *Helicoverpa zea* at 0.025, 0.05 and 0.1% (W/V) (Young *et al*., 1995). Chen *et al*. (2007) cited that tannic acid has a lethal effect against the cotton bollworm *H. armigera* due to its activity as the most potent inhibitor of the bollworm enzymes. This acid also showed inhibition activity against the growth of the pink bollworm *P. gossypiella* larvae. In addition, tannic acid caused cytotoxicity of murine B16 - F10 melanoma cell line with LC_{50} of 7 micro M and complete lethality was observed at 20 micro M. The sensitivity of insects to tannic acid may be a consequence of its extensive chemical modification in the midgut and oxidation is the first thinkable chemical modification (Kubo *et al*., 2008**)**.

Table (4): Toxic effect of tannic acid on the 1st instar larvae of *E. insulana* and *P. gossypiella* at laboratory conditions

Insect species	Conc. (ppm)	Mortality % at indicated days 1	3	6	9	13
E. insulana	2000	51.67^{a}	53.33^{a}	35.00^{a}	64.00^{a}	70.00^{a}
	1500	43.67^{b}	43.33^{b}	53.33^{ab}	62.00^{a}	65.00^{b}
	1000	41.67^{b}	43.33^{b}	55.00^{a}	60.00^{a}	63.00^{b}
	500	30.00^{c}	41.66^{b}	50.00^{ab}	53.00^{b}	62.00^{b}
	250	28.33^{c}	40.00^{b}	48.00^{b}	51.00^{b}	62.00^{b}
	Control	5.00^{d}	5.00^{c}	10.00^{c}	10.00^{c}	10.00^{c}
	F. test	**	**	**	**	**
	L.S.D.$_{0.05}$	6.97	6.62	6.97	6.41	4.65
P. gossypiella	2000	26.66^{a}	35.00^{a}	41.66^{a}	45.00^{a}	45.00^{a}
	1500	21.66^{b}	33.00^{ab}	36.33^{ab}	38.00^{b}	42.00^{a}
	1000	18.33^{bc}	28.33^{bc}	33.33^{b}	36.66^{bc}	40.00^{ab}
	500	15.00^{c}	25.00^{c}	31.66^{b}	35.00^{bc}	36.66^{b}
	250	8.33^{d}	18.33^{d}	25.00^{c}	31.66^{c}	31.66^{c}
	Control	5.00^{d}	5.00^{e}	5.00^{d}	10.00^{d}	10.00^{d}
	F. test	**	**	***	***	***
	L.S.D.$_{0.05}$	4.48	8.63	5.64	6.17	5.05

The same letter in the same column means not significant at P < 0.05

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تأثير تريكوديرما هارزيانم و بعض الأحماض العضويه على ديدان لوز القطن إيرياس إنسيولانا و بكتينوفورا جوسيبيلا

سالم عبد الفتاح أحمد المصري ، هند شكري غريب و محمد السيد محمد حجاب

معهد بحوث وقايه النباتات – الدقي – جيزه – مصر

أجريت الدراسه المعمليه بهدف معرفه تأثير فطر تريكوديرما هارزيانم في صورتين الراشح و الجراثيم على الطور اليرقي الأول لديدان لوز القطن الشوكيه إيرياس إنسيولانا و القرنفليه بكتينوفورا جوسيبيلا كما إمتدت الدراسه أيضا إلى دراسه التأثير السام لحمضين السالسيلك و التانيك على نفس النوعين من اليرقات. و قد أوضحت النتائج أن نسبه موت اليرقات من النوعين تتناسب طرديا مع معدلات الراشح و تركيزات جراثيم الفطر حيث تسبب راشح فطر التريكوديرما هارزيانم بعد ثلاث أيام من العدوى و عند جميع معدلاته ٠,٢٥ و ٠,٥ و ١ ملل في موت ٦٠٪ من يرقات إيرياس إنسيولانا كما حققت نفس هذه المعدلات موت ٤٠ و ٣٣,٣٣ و٣١,٦٧٪ من يرقات بيكتينوفورا جوسيبيلا بالتتابع. بينما بعد نفس الفتره من العدوى سجلت تركيزات الفطر ٢× ١٠^{٥} و ١× ١٠^{٥} و ٠,٥× ١٠^{٥} جرثومه / ملل موت ٦٠ و ٣٣, ٥٣ و٥٠٪ للإيرياس إينسيولانا و ٨٠ و ٧٦ و ٧٥٪ موت للبيكتينوفورا جوسيبيلا على التوالي. و فيما يخص حمض السالسيلك فقد أظهرت النتائج أنه حقق بعد ١٣ يوم من المعامله موت ٥٠ و ٤٨ و٤٦ و ٤٥ و٤٥٪ للإيرياس إينسيولانا و ٨٦,٦٧ و ٨٣ و ٨٠ و ٧٨ و ٧٦,٦٧ للبيكتينوفورا جوسيبيلا عند التركيزات ١٩٠٠ و ١٤٢٥ و ٩٥٠ و ٤٧٥ و ٢٣٧,٥جزء من المليون بالتتابع. بينما سجل حمض التانيك بعد ١٣ يوم من المعامله موت ٧٠ و ٦٥ و٦٣ و ٦٢ و٦٢٪ من الإيرياس إنسيولانا و ٤٥ و ٤٢ و٤٠ و ٣٦,٦٦ و ٣١,٦٦٪ موت للبيكتينوفورا جوسيبيلا عند التركيزات ٢٠٠٠ و ١٥٠٠ و ١٠٠٠ و ٥٠٠ و ٢٥٠ جزء من المليون على التوالي.