The entomotoxicity of Destruxin and Nano-Destruxin against three olive pests under laboratory and field conditions

Sabbour M.M[1] and S.M. Singer[2]

1.Department of Pests and Plant Protection,2.Vegetabe research Dep. National Research Centre, Dokki, Cairo, Egypt

E-mail: sabbourm@yahoo.com Tel.: +202/01223305136 Fax: +202/33370931

ABSTRACT

The toxin of the fungus *Metarhizium anisopliae* , Destruxin and Nano- Destruxin was tested against the olive insect pests: *Bactrocera oleae*, *Ceratitis capitata* and *Prays oleae* under laboratory and field conditions.

The half lif period, LC50 of the three serious olive pests under laboratory conditions after Destruxin treatments , which show that, *B. oleae* LC50 obtained 110 mg/L. the LC50 of *C. capitate and B. oleae* recorded 121 and 132mg/L respectively.

When the different concentrations of nano- Destruxin were evaluated against the three olive insect pest the LC50 obtained 66, 69 and 71 mg/L , for *P. oeae*, *C. capitata* and *B. oleae*, respectively .

Under field conditions during season 2013, the infestations of the three olive pests were recorded, the lowest percent of infestations. The means number after 120 day of applications recorded, that the three pests *B. oleae , C. capitata* and *P.oleae* were significantly decreased to 10±4.2 , 13±2.3 and 22±2.3 10±4.2 individuals as compared to 89±1.2, 92±4.2 and 91±4.0 individuals in the control. Also, the means number of infestations were significantly decreased after nano-Destruxin treatments during 2014.

Results , show that ,during season 2013 at the harvest time , the weight of olive fruits recorded, 3996± 20.90 and 4599± 50.90 kg/feddan in the trees treated with Destruxin and Nano-Destruxin, respectively as compared to 2020± 20.72 kg/ feddan in the control. During season 2014, the weight of olive fruits were significantly increased to 4094±71.58 and5431± 20.70 after Destruxin and Nano- Destruxin treatments as compared to 1901±89.30 kg/feddan in the control.

Key words: *Bactrocera oleae*, *Ceratitis capitata*, *Prays oleae* ,*Destruxin , nano.*

INTRODUCTION

Olive (*Olea europaea* L.) has become one of the important economical crops in Egypt. Its cultivated area has been expanded largely in the last decade, particularly in new reclaimed arid areas (Western side of the Nile). Egypt cultivated 125000 feddans that exceeded 15 million olive trees. An estimated production of 450 000 ton of olives (sfi-egypt.com/about.asp, 2010). Olive tree is subjected to attack by many insect pests that affect yield quality and quantity. Among the most common pest species surveyed in Egypt are: *Bactrocera oleae* (Rossi), *Prays oleae* (Bern.) and *Ceratitis capitata* (Wied.), *Key* of damaging olive trees is *B. oleae* (Rice, 2000 and Eid, 2003).

P. oleae is one of the most important insect pests of olives in Egypt and other Mediterranean countries. The moth develops three generations per year (El-Basha,

2002). In Egypt the first generation of moths appears in April the female lays its eggs on the flower buds, the newly hatched larvae feed on the buds and flowers (El-Basha, 2002). The Mediterranean fruit fly *C. capitata* (Wiedermann) and the olive fruit fly *B. oleae* (Gmelin) (Diptera: Tephritidae) are from the serious insect pests which attack the olive fruits and cause an economical destruction to the olive trees. These pests were controlled by chemical insecticides which pollute the environment and causes cancer diseases, where bioinsecticides could control these pests safely (Roberts and Humber, 1981; Tanda and Kaya, 1993; Hajek and St. Leger, 1994).

Destruxin capacity in control practices is a less studied matter, but some investigations have described its insecticidal properties (Brousseau et al., 1996; Thomsen and Eilenberg, 2000). In this study different dilutions of destruxins extracted from several fungal isolates were examined on citrus leafminer larvae.

The present study aims to evaluate the pathogenicity of the isolates the entomopathogenic fungus, *Destruxin* and nano- Destruxin against three serious olive pests under laboratory and field conditions. It is necessary to find alternative safety insecticides to reduce the heavy doses of chemical insecticides which is used for olive pests control.

MATERIALS AND METHODS

Laboratory tests:

Insects:

B. oleae and *C. capitata* adults used in the present work were obtained from laboratory colonies maintained in our laboratory at 25±2°C and 60–65% relative humidity (RH) and 12:12 (L:D) photoperiod. Adults were provided with water and a solid diet consisting of 40% sugar, 10% hydrolyzed yeast, 5% egg yolk. The olive Moth, *Prays oleae* (Bernard, 1788) (Lepidoptera: Yponomeutidae), was reared on olive leaves under the same laboratory conditions. Adults reared in cylinder glass cages (15cm diameter x 22cm height), covered with muslin, and fed on 10% sucrose solution.

Isolation of the fungi:

The fungus *Destruxin* was isolated from the diseased insect pests (*C. Capitata*, *B. oleae*& *P. oleae*). Isolates were subcultured on nutrient PDA medium. Isolates were identified at National research Centre (NRC) Plant Pathology Department. The spores of *I. fumosorosea*, were collected from agar surface of the fungus culture in 15cm diameter Petri-dish. Spore suspension in water + 0.1% Tween-80 was prepared. The strength of original culture was $1x10^8$ spore/ml. It was used as stock suspension and kept in a refrigerator at 4°C. From this stock, dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by

culturing the fungus on liquid medium in 1 L cellculture glass bottles according to Rombach *et al*., (1988) and modified by El-Husseini *et al*. (2004).

Bioassays against target pests:

All fungal isolates concentrations of *Destruxin and nano- Destruxin*, ranged from $1x10^2$ to $1x10^8$ spores/ml were prepared by 1-10 fold dilution from the main stock culture ($1x10^8$) and tested under controlled conditions (25±2ºC and 65±5% RH) against *C. Capitata, B. oleae& P. oleae* adults. Ten 3-day-old flies were collected in test tubes, immobilized on ice and carefully transferred to PDA dishes (9 cm diameter) containing the six fully developed fungal colonies. The flies were allowed to walk on the fungal colonies for 5–10 min depending on fly mobility until the flies collected spores on their body. The flies were then removed from the Petri dishes and placed in small cages (10 cm x 10 cm x 10 cm). The same number of flies treated similarly but with uninoculated PDA plates was used as controls. Solid diet and water were offered to flies and kept under rearing conditions. Dead flies were counted and removed from the cages daily for 21 days. Each treatment was replicated five times The percentages of mortality were calculated after seven days and corrected according to Abbott's formula (Abbott, 1925), while the LC50 value was calculated through Probit analysis according to Finney equation (Finney, 1971).

Field experiments:

Esraa village- El-Nobaryia region, during the two successive seasons 2011&2012 starting from the first of July till the end of August to evaluate the efficacy of the tested fungi against the target insect pests under field conditions. Three random patches of Olive trees were selected, each comprised 12 trees (12 trees for *Destruxin* applications and 12 trees for control) to carry out the field experiment. *I. fumosorosea*, nano- Destruxin was applied, each as a single treatment at the rate of $1x10^8$ spores/ml. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at the sunset with a ten litre sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of the application. Each treatment was replicated four times. Four plots were treated with water as control. Random samples of leaves and fruits olives plants were weekly collected from each treatment and transferred to laboratory for examination. The infestation of *C. capitata, B. oleae& P. oleae* were estimated in each case.

After harvest, yield of each treatment was weighted as Kg/Feddan.

RESULTS

Table 1 show that the LC50 of the three serious olive pests under laboratory conditions after Destruxin treatments , which show that, *B. oleae* LC50 obtained 110 mg/L. the LC50 of *C. capitate and B. oleae* recorded 121 and 132mg/L respectively. (Table 1)

When the different concentrations of nano- Destruxin were evaluated against the three olive insect pest the LC50 obtained 66, 69 and 71 mg/L , for *P. oeae, C. capitata* and *B. oleae*, respectively (Table 2)

Under field conditions during season 2013, the infestations of the three olive pests were recorded, the lowest percent of infestations. The means number after 120 day of applications recorded, that the three pests *B. oleae , C. capitata* and *P. oleae* were significantly decreased to 10±4.2 , 13±2.3 and 22±2.3 10±4.2 individuals as compared to 89±1.2, 92±4.2 and 91±4.0 individuals in the control (Table 3). Also, the means number of infestations were significantly decreased after nano-Destruxin treatments during 2014.

Table 4, show that ,during season 2013 at the harvest time , the weight of olive fruits recorded, 3996± 20.90 **and** 4599± 50.90 **kg/feddan in the trees treated with** Destruxin and Nano- Destruxin, respectively as compared to 2020± 20.72 kg/ feddan in the control. During season 2014, the weight of olive fruits were significantly increased to 4094±71.58 and5431± 20.70 after Destruxin and Nano- Destruxin treatments as compared to 190**1**±89.30 kg/feddan in the control.

figure 1 show that the toxin treatments and nano Destruxin leads to the infestations decrease during season 2013. During season 2014 the infestations with the three serious olive pests were significantly decreased as compared to control (figure 2)

The obtained results are similar to other studies carried out by Castillo *et al.* (2000) and Espin*et al*. (1989) on their work on*C. capitata*. After harvest the olive fruits weight were 2598 ±3 0.30 Kg/Feddan in the plots treated with *Destruxin* compared to 2200± 20.72 Kg/Feddan in the control during season 2011(Tabe 3). During season 2012 the treatments trees with *Destruxin scored the highest weight* 3890±75.37Kg/ feddanas compared to 1999± 86.50 Kg/feddan among the control trees. In all cesses, during the both seasons 2011 and 2012 the yield loss ranged between 38.85 and 48.61 % in the control (Table 3). These results agree with Sabbour &Shadia Abd El-Aziz, (2002 and 2010) and Shadia Abdel Aziz &Nofel (1998), who proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests. Also, results were in accordance with Castillo *et al.* (2000) who reported that the virulence of *B. bassiana* against *C. capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits. Espin *et al*. (1989) recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments. Konstantopoulou and Mazomenos (2005) reported that the fungi *B. bassiana* and *B. brongniartii* application considered the most pathogenic to *C. capitata* causing 97.4 and 85.6% mortality, while *M. anisopliae* cause a highly mortality rates to *C. capitata* and *B. oleae* adults and the rate of larval mortality was 85.2%. In Egypt, Mohamed (2009) reported that the fungi *Lecanicillim lecanii, M. anisopliae* and inter action between *B. bassiana* and *M. anisopliae* are suitable candidates to be used for control of *P. oleae*. Abdel-Rahman & Abdel-Mallek (2001), Abdel-Rahman (2001) and Abdel-Rahman *et al*. (2004), controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab (2005, 2007), Sabbour and Shadia Abd El-Aziz (2002 and 2010) and Sahab and Sabbour (2011) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.

Acknowledgment

This research was supported by Agric. Department, National Research Centre, Cairo, Egypt. Project No (10120601).

Conclusions

The usage of the microbial control agent toxin (Destruxin and nano-Destruxin) are more effective for controlling the three serious olive pest under laboratory and field conditions

Table (1): Effect of Destruxin on the target insect pests under laboratory conditions

Target pests	LC_{50} (mg/L)	Slope	Variance	95% Confidence limits
Prays oleae	110	0.01	0.02	177-245
Ceratitis capitata	121	0.01	0.03	188-277
Bactrocera oleae	132	0.02	0.01	200-278

Table (2): Effect of nano-Destruxin on the target insect pests under laboratory conditions

Target pests	LC_{50} (mg/L)	Slope	Variance	95% Confidence limits
Prays oleae	66	0.01	0.02	37-95
Ceratitis capitata	69	0.01	0.03	28-97
Bactrocera oleae	71	0.02	0.01	55-78

Table 3. Infested plants with target insect pests after treatment with the fungi toxin Destruxin and nano- Destruxin **under field conditions through out the two successive seasons**

Treatment	Days after treatment	El-Esraa (Nobaryia) Number of infestations ±S.E					
		Season 2013			Season 2014		
		B. oleae	*C. capitata*	*P.oleae*	***B. oleae***	***C. capitata***	***P.oleae***
Control	20	14.1±5.1	14.1±9.1	1 3.9±1.4	22.1±2.5	5.4±2.3	6.9±2.9
	50	26±2.8	25±2.3	24±.2	29±2.2	27±3.4	22±3.4
	90	44±4.4	43±2.4	46±5.4	49±3.6	49±3.7	59±4.6
	120	89±1.2	92±4.2	91±4.0	89±1.2	93±3.3	99±6.9
Destruxin	20	0±0.0	1.1±1.2	0±0.0	1.5±2.1	2.4±5.3	1.1±3.9
	50	4±2.2	5±3.1	6±2.2	2±4.5	8±4.4	5±3.4
	90	11±4.1	11±3.7	10±3.2	10±3.4	11±3.4	9±3.7
	120	12±4.2	16±2.3	12±2.3	12±3.5	14±2.9	10±4.5
Nano-Destruxin	20	0±0.0	1.1±1.2	0±0.0	1.5±2.1	2.4±5.3	1.1±3.9
	50	4±2.2	5±3.1	6±2.2	2±4.5	10±4.4	9±3.6
	90	11±4.1	10±3.7	17±3.2	10±3.4	11±9.4	11±3.8
	120	10±4.2	13±2.3	22±2.3	9±3.5	12±2.9	13±4.5
F value	37.0	5	8	5	17	23	20
Lsd5%	11.7	2	8	6	11	11	10

Table (4): Weight of harvested olive fruits after treatment with the Destruxin against target insect pests during two successive seasons.

Treatment	Weight of yield in El-Esraa (Nobaryia) Season 2013	Season 2014
	Kg/Feddan	Kg/Feddan
Control	2020± 20.72	1901±89.30
Destruxin	3996± 20.90	4094±71.58
Nano-Destruxin	4599± 50.90	5431± 20.70
F-value	35.11	34.8
LSD 5%	83	86

Fig 1. Infestations of olive three insect pests in the field during season after the fungi toxin treatments 2013

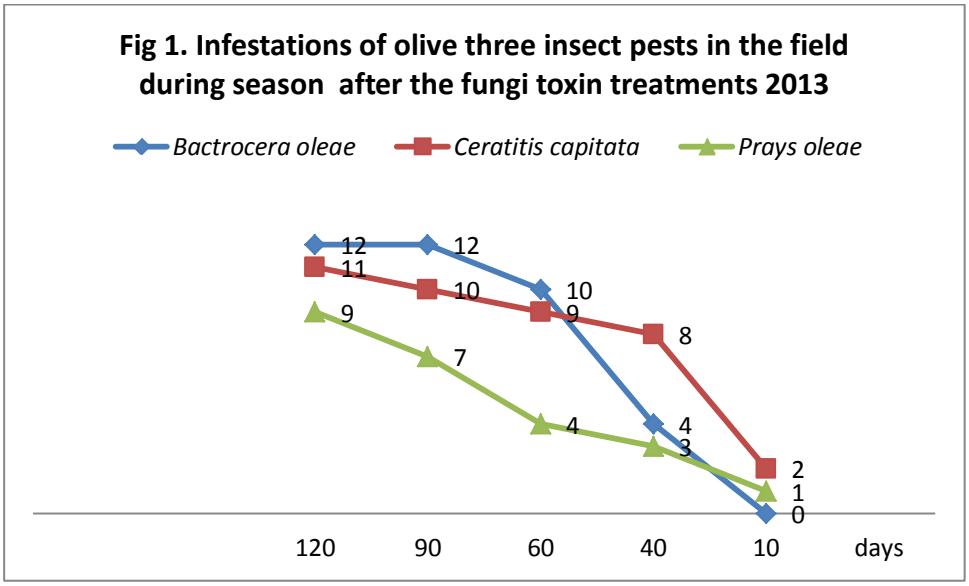
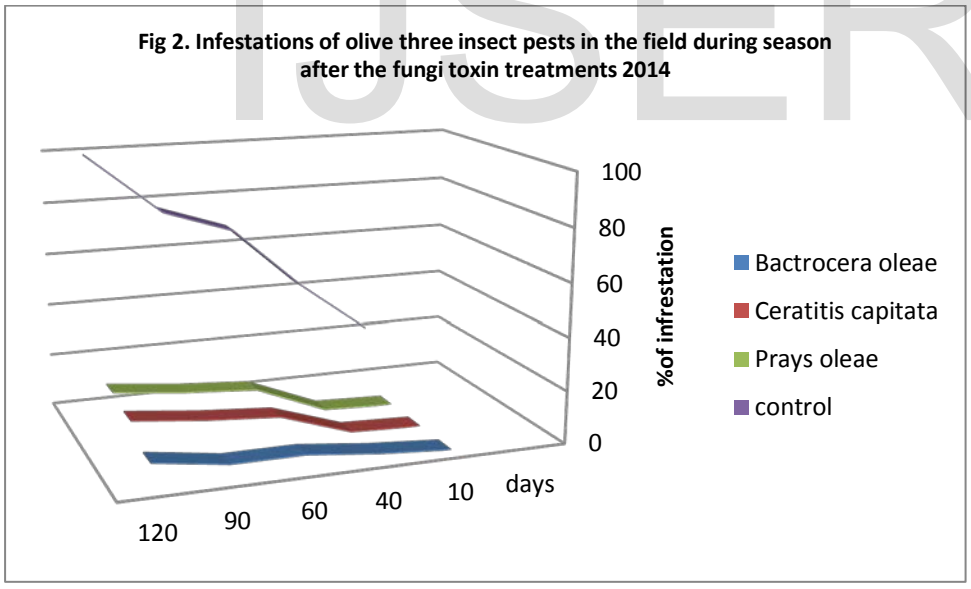


Fig 2. Infestations of olive three insect pests in the field during season after the fungi toxin treatments 2014.



REFERENCES

Abbott, W.W. (1925). A method of computing the effectiveness of an insecticide. J. Economic Entomol.,18: 265-267.

Abdel-Rahman, M.A.A. (2001). Seasonal prevalence of entomo-pathogenic fungi attacking cereal aphids infesting wheat in southern Egypt. Inter. Symposium. Agric. Agadir-Morocco, 7-10: 381-389.

Abdel-Rahman, M.A.A and Abdel-Mallek, A.Y. (2001).Paramilitary records on entomopathogenic fungi attacking cereal aphids infesting wheat plants in southern Egypt. First Conference for safe Alternatives to pesticides for pest managements, Assiut: 183-190.

Abdel-Rahman, M.A.A.; Abdel-Mallek, A.Y.; Omar S.A. and Hamam, A.H. (2004).Natural occurrence of entomopathogenic fungi on cereal aphids at Assiut. A comparison study between field and laboratory observations. Egypt. J. Boil. Sci., 14: 107-112.

Brousseau, C., G. Charpentier and S. Belloncik, 1996. Susceptibility of spruce budworm, *Choristoneura funiferana* Clemens, to destruxines, cyclopeptidic
mycotoxins of Metarhizium anisopliae. J. Invertebr. Pathol., 68: 180-182.

Castillo, M.A.; Moya, P.; Hernandez, E. and Primo-Yufera, E. (2000). Susceptibility of *Ceratitis capitata* Wiedenmann (Diptera: Tephritidae) to entomopathogenic fungi and their extract. Biol. Cont., 19: 274-282.

Eid, F.M. (2003). Survey of the insect pests infesting olive with reference to the olive fruit fly, *Bactrocera oleae* Gmel and parasitoid in North Simi. J. Agric. Sci. Mansoura Univ., 28: 8461-8469.

El-Husseini, M.M.; Shahira, S. Marie; A.M. Amal; A. El-Zoghby; Sahar S. Ali, Naglaa, A.M. Omar; E.A. Agamy; H.E. Abou Bakr; M.S. Nada; Sherin Tamer; Hannah, M. Kamal and A.M. Ibrahim (2004). Isolation, Production and use of entomopathogenic fungi for controlling the sugar beet insect pests in Egypt. Egypt. J. Biol. Pest Control., 14(1): 265-275.

Espin, G.A. T. laghi De .S.M., Messias, C.L. and Pie-Drabuena, A.E. (1989).Pathogencidad de *Metarhizium anisopliaenas* diferent esfases de desenvolvim ento de *Ceratitis capitata* (Wied.) (Diptera: Tephritidae). Revista Brasileria de Entomologia, 33: 17-23.

Finney, D.J. (1971). Probit Analysis, Cambridge: Cambridge University Press.

Hajek, A.E. and R.J. St. Leger. (1994). Interactions between fungal pathogens and insect hosts. Annu. Rev. Entomol., 39: 293-322.

Konstantopoulou, M.A. and B.E. Mazomenos (2005).Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. Bio Control, 50(2): 293-305.

Mohamed, F.M. (2009). Pathogenicity of three commercial products of entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* against adult of olive fly *Bactrocera oleae,* (Gmelin) (Diptera: Tephirtidae) in the laboratory. Plant Prot. Sci., 3: 98-102.

Montiel, A and Jones, O. (2002). Alternative methods for controlling the olive fly *Bactrocera oleae,* involving semio chemicals. IOBC wprs Bull., 25: 1-11.

Qiao, Meihua; Daniel E. Snyder, Jeffery Meyer, Alan G. Zimmerman, Meihau Qiao, Sonya J. Gissen danner, Larry R. Cruthers, Robyn L. Slone, Davide R. Young (2007). Preliminary Studies on the effectiveness of the novel pulicide, spinosad, for the treatment and control of fleas on dogs. Veterinary Parasitol., 345–351. Retrieved 3 May 2012.

Rice, R.E. (2000). Bionomics of the olive fruit fly *Bactrocera* (*Dacus*) *oleae.* Univ. of California Plant Prot. Quart, 10:1-5.

Roberts, D.W. and R.A. Humber (1981).Entomogenous fungi In Biology of Conidial Fungi (G.T. Cole and W.B. Kendrick, eds), vol. 2, pp. 201-236. Academic Press, New York.

Rombach, M.C.; Aguda, R.M. and Robert, D.W. (1988).Production of *Beauveria bassiana* in different liquid media and subsequent conditions mycelium. Entomol., 33: 315-234.

Sabbour, M.M. and Shadia E. Abed El-Aziz (2002). Efficacy of some botanical oils formulated with microbial agents against the cotton leafworm and greasy cutworm attaching cotton plants. Bull. Ent. Soc. Egypt. ser. 28, 2001-2002: 135-151.

Sabbour, M.M. and Sahab, A.F. (2005).Efficacy of some microbial control agents against cabbage pests in Egypt. Pak. J. Biol. Sci., 8: 1351-1356.

Sabbour, M.M. and Sahab, A.F. (2007).Efficacy of some microbial control agents against *Agrotis ipsilon* and *Heliothis armigera* in Egypt. Bull. N.R.C. Egypt. 13

Sabbour, M.M. and Shadia, E. Abd-El-Aziz (2010).Efficacy of some bioinsecticides against *Bruchidius incarnates* (BOH.) (Coleoptera: Bruchidae) Infestation during storage. J. Plant Prot. Res., 50 (1): 28-34.

Sahab, A.F. and Sabbour, M.M. (2011).Virulence of four entomo-pathogenic fungi on some cotton pests with especial reference to impact of some pesticides, nutritional and environmental factors on fungal growth. Egypt. J. Boil. Pest Cont., 21 (1): 61-67.

Shadia E. Abdel Aziz and Nofel, M.A. (1998). The efficacy of bacteria, fungi and natural products in baits against the greasy cutworm *Agrotisipsilon* (Hufn.) (*Lepidoptera*: *Noctuidae*) in Egypt. J. Egypt. Ger. Soc. Zool., 27. Ent. 129-139.

Tanda, Y. and Kaya, H.K. (1993).Insect Pathology. Academic Press, San Diego, CA, USA.

Thomsen, L. and J. Eilenberg, 2000. Time concentration mortality of *Pieris brassicae*
(Lepidoptera: Pieridae) and *Agrotis segetum* (Lepidoptera: Noctuidae) larvae from different destruxines. Environ. Entomol., 29: 1041-1047.