J. Agric. Sci. Mansoura Univ., 30 (1): 603 - 609, 2005

EVALUATION OF SOME HONEY BEE PRODUCTS AND CHEMICAL COMPOUNDS AGAINST FUNGAL PATHOGENS OF HONEY BEE COLONIES

Ali, M.A.M.[1] and N.Y. Abd El-Ghafar[2]

1- Department of plant protection, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

2 Department of plant pathology, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

ABSTRACT

Application of chemical compounds (i.e. Moncerin, Rhizolex T and Topsin M) and bee products (i.e. bee honey, propoils and royal jelly) were reduced growth of *Ascosphaera apis* (Chalk brood disease of honey bee) and *Aspergillus flavus* (stone brood disease of honey bee), compared with the control. Percentage of growth reduction to both pathogens was increased with increasing the rates of chemical compounds or bee products application. Meanwhile, application of chemical compounds were more effective than naturally products to reduction growth of *A. apis* and *A. flavus.* However, royal jelly as naturally products and topsin M as chemical compounds were the most effective to reduction growth of both pathogens. Application of both Rhizolex T or Moncerin as chemical compounds and propolis or bee honey as bee products were moderately effective.

Keywords: Honey bee products, Chemical compounds, *Ascosphaera apis,* Chalk brood disease, *Aspergillus flavus*, Stone brood disease, Hone bee colonies

INTRODUCTION

Chalk brood and Stone brood diseases caused by *Ascosphaera apis* and *Aspergillus flavus*, respectively. Larvae were most susceptible to *A. apis*. When they were 3- 4 days old and were chilled briefly two days later immediately after sealing in their cells to pupate. While, larvae were most die by *A. flavus* after they have been capped in their cells prior to pupation (Bailey, 1967). Often fungal spores germinate on the cuticle of larvae and sometimes the mycelium penetrate the sub-cuticle tissue and produce local hyphae and conidiphores, but the infection is usually via the gut (Roussy, 1962).

Addition of sorbic acid and Sodium propionate in pollen supplement or replacement of old combs with new ones (Nelson and Gochnauer, 1982) and antibacterial compounds (Yatsumami and Echigo, 1984) reduced larval infection. In the available literature several attempts were made to use honey bee products for controlling fungal diseases in the honey bee colonies such as Royal jelly (Tamura *et al* 1985; Bamford, 1987; Chiu & Chu, 1990; Abd Alla *et al* 1995, Xiao *et al* 1996; Hornitzky, 1998; Okuda *et al* 1998 and Tomoko et al., 1998) and bee glue, the so-called propolis (Brumfitt et al., 1990; Langoni *et al* 1996 and El-Dieb *et al* 1997).

Osman *et al* (1987) in Egypt were the first who mentioned that propolis extract may be used as anti phytopathogenic fungi. They added that

promising results were obtained when it was used against several fungi belonging to the major classes of phytopathogens, i.e. Oomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes. In Saudi Arabia Kingdom (Abdulsalam and Mohamed, 1989) who is the first record to test the antifungal activity of local propolis.

The present work aimed to study the effects of bee products (i.e. royal jelly, bee honey and propolis) as naturally products and chemical compounds (i.e. Topsin-M, rhizolex T and Mencerin), at different rates, on growth of *A. apis* and *A. flavus* in vitro.

MATERIAL AND METHODS

1. Fungi

Ascosphaera apis and *Aspergillus flavus* were isolated and identification from infected honey bee larvae **(Ali, 2003).** These larvae were collected from honey bee colonies, at different times and from several locations.

2. Bee products and chemical compounds

Bee honey, propolis and royal jelly were applied as naturally products. These products were used at rate of, 1, 3 and 5 g / liter. Meantime, Topsin-M (70% wp), Rhizolex T (50 wp) and Mencern (50% wp) were used as chemical compounds. These compounds were applied at rate of 0, 0.5, 1.0 and 1.5 g/L.

3. Effect of bee products and chemical compounds on growth of *A. apis* and *A. flavus*, in vitro

The effect of different rates of tested bee products or chemical compounds on growth of *A. apis* and *A. flavus* were studied on potato dextrose agar (PDA) medium according to Abdulsalam and Mohamed (1989). Check treatment was carried out on PDA medium without products or compounds. All products or compounds were added to PDA medium before solidifying and rotated gently to ensure an equal distribution. Treated medium was poured in sterilized Petri-dishes. Inocula (5 mm diameter) of *A. apis* and *A. flavus* were taken from 7 days-old culture and transferred singly to the centre of the dishes. Four dishes were used as replicates for each rate of products or compounds. Inoculated dishes were incubated at 30 C. Average of diameter linear growth was measured at 4, 8 and 12 days from the inoculation and the percentage of growth reduction (PGR) were calculated in relation to the control treatment as the following.

$$PGR = \frac{growth\ in\ control - growth\ in\ treatment}{growth\ in\ control}\ X\ 100$$

Data were statistically analyzed using the "F" test and the value of LSD ($P \leq 0.05$) was calculated according to Cochran and Cox(1957)

RESULTS AND DISCUSSION

Effect of bee products and chemical compounds on:
a. Growth of *A. apis*

Data in Tables (1 and 2) indicate that application of bee honey or propoils or royal jelly as naturally products and Moncerin or Rhizolex-T or Topsin M as chemical compounds were decreased growth of *A. apis* compared with the control.

Table 1. Influence of different bee products at different rates on growth of *Ascosphaera apis* in vitro

Treatment	Rate g/L	Average of diameter growth (cm)			Growth reduction (%)		
		4 days	8 days	12 days	4 days	8 days	12 days
Bee honey	1	1.6	3.9	6.7	23.8	11.4	9.5
	3	1.4	3.7	6.5	33.3	15.9	12.2
	5	1.1	3.4	6.2	47.6	22.7	16.2
Propolis	1	1.2	3.5	6.3	42.9	20.5	14.9
	3	1.0	3.2	6.0	52.4	27.3	18.9
	5	0.7	2.7	5.4	66.7	38.6	27.0
Royl jelly	1	1.5	3.8	6.6	28.6	13.6	10.8
	3	1.3	3.5	6.3	38.1	20.5	14.9
	5	1.0	3.0	5.8	52.4	31.8	21.6
Control	0	2.1	4.4	7.4	0.0	0.0	0.0

LSD at 5%

Treatment	**: 0.6**	**Rate**	**: 0.2**
Time	**: 1.2**	**Interaction**	**: 3.0**

Table 2. Influence of different chemical compounds at different rates on growth of *Ascosphaera apis* in vitro

Treatment	Rate g/L	Average of diameter growth (cm)			Growth reduction (%)		
		4 days	8 days	12 days	4 days	8 days	12 days
Moncerin	0.5	1.4	3.5	6.6	33.3	20.5	10.8
	1.0	1.2	3.3	6.3	42.9	25.0	14.9
	1.5	0.9	2.9	5.9	57.1	34.1	20.3
Rhizolex	0.5	1.2	3.2	6.1	42.9	27.3	17.6
	1.0	1.0	3.0	5.8	52.4	31.8	21.6
	1.5	0.6	2.6	5.3	71.4	40.9	28.4
Topsin M	0.5	0.8	2.8	5.6	61.9	36.4	24.3
	1.0	0.6	2.5	5.2	71.4	43.2	29.7
	1.5	0.3	2.0	4.6	85.7	54.5	37.8
Control	0.0	2.1	4.4	7.4	0.0	0.0	0.0

LSD at 5%

Treatment	**: 0.4**	**Rate**	**: 0.2**
Time	**: 1.0**	**Interaction**	**: 2.7**

Percentage of growth reduction was increased with increasing the rates of products and compounds application. Application of chemical compounds were more effective than bee products to reduction growth of *A. apis*, where percentage of growth reduction was 10.8 - 85.7% and 9.5 – 52.4%, respectively. Efficiency of bee products and chemical compounds were decreased with increased period test.

Royal Jelly as naturally product was the most effective to growth reduction of *A. apis*, where percentage of growth reduction was ranged from 10.8 to 52.4 at the rate of 1,5 g/L. Meantime, bee honey and propoils were moderately effective. However, topsin-M at rate of 1.5 g/L was the most effective to growth reduction of *A. apis,* where percentage of growth reduction was ranged from 24.3 to 85.7% and Rhizolex-T and Moncerin were less effective.

b. Growth of *A. flavus*

Application of previously bee products or chemical compounds were reduced growth of *A. flavus* compared with the control. Percentage of growth reduction was increased with increasing the rates of products or compounds application. Application of chemical compounds were more effective than bee products to growth reduction of *A. flavus*, where percentage of growth reduction was 23.3 – 87.1 and 16.7 – 71.0%, respectively. Application of royal jelly as naturally products was the most effective to growth reduction, where percentage of growth reduction was ranged from 22.2 to 71.0% at the rate of 5 g/L. Meanwhile, bee honey and propoils were moderately effective. However, topsin-M as chemical compounds was the most effective to growth reduction, where percentage of growth reduction was ranged from 46.7 to 87.1% at the rate of 1.5 g/L and rhizolex-T and mocerin were less effective (Table 3 and 4).

Table 3. Influence of different bee products at different rates on growth of *Aspergillus flavus* in vitro

Treatment	Rate g/L	Average of diameter growth (cm)			Growth reduction (%)		
		4 days	8 days	12 days	4 days	8 days	12 days
Bee honey	1	2.0	4.9	7.5	35.5	25.8	16.7
	3	1.7	4.6	7.3	45.2	30.3	18.9
	5	1.3	4.0	6.9	58.1	39.4	23.3
Propolis	1	2.2	5.2	7.8	29.0	21.2	13.3
	3	1.9	4.9	7.5	38.7	25.8	16.7
	5	1.6	4.2	7.1	48.4	36.4	21.1
Royl jelly	1	1.5	4.0	7.0	51.6	39.4	22.2
	3	1.2	3.7	6.7	61.3	43.9	25.6
	5	0.9	3.1	6.1	71.0	53.0	32.2
Control	0	3.1	6.6	9.0	0.0	0.0	0.0

LSD at 5%
Treatment : 0.5 **Rate : 0.2**
Time : 1.4 **Interaction : 2.9**

Table 4. Influence of different chemical compounds at different rates on growth of *Aspergillus flavus* in vitro.

Treatment	Rate g/L	Average of diameter growth (cm)			Growth reduction (%)		
		4 days	8 days	12 days	4 days	8 days	12 days
Moncerin	0.5	1.7	4.4	6.9	42.2	33.3	23.3
	1.0	1.5	4.0	6.5	51.6	39.4	27.8
	1.5	1.1	3.5	6.0	64.5	47.0	33.3
Rhizolex	0.5	1.3	3.6	6.0	58.1	45.0	33.3
	1.0	1.0	3.3	5.5	67.7	50.0	38.9
	1.5	0.9	2.9	4.9	70.9	56.1	45.6
Topsin M	0.5	0.9	3.0	4.8	71.0	54.5	46.7
	1.0	07	2.5	4.4	77.4	62.1	51.1
	1.5	0.4	1.9	3.9	87.1	71.2	56.7
Check	0.0	3.1	6.6	9.0	0.0	0.0	0.0

LSD at 5%
Treatment : 0.8 **Rate : 0.3**
Time : 1.4 **Interaction : 3.2**

A new potent antibacterial protein, the 50-called royalisin was found in royalisin was found in royal jelly of the honey bee this protein is an amphipathic protein and showed extensive sequence homology to two other antibacterial proteins, sapecin from embryonic *Sarcophaga peregnna* cells and phormicins from *Phomia terranovae* larvae (Fujiwar *et al* 1990).

Tamura *et al* (1985) mentioned that royal jelly caused damage of DNA in the nucleu of the fungi of may cause number of mutation in the experimental fungi which lead to complete inhibition and final death of fungi. Bamford (1987) stated that royal jelly exhibited a severe inhibition effect on all germination stages of *A. apis.* Chu *et al* (1992) found that presence of 10-hydroxydecanoic acid (10-HDA) in royal jelly plays an important in inhibiting growth or promoting sporulating of *A. apis*. Brumfitt *et al.* (1990) reported that bee glue showed a weak inhibition activity in vitro against certain species of gram positive bacteria. However, Abdulsalam & Mohamed (1989); Langoni *et al* (1996) and El-Dieb *et al* (1997) found that propoils caused complete inhibition of growth in many microorganisms species.

REFERENCES

Abd Alla, M.S.; A. Mishref and I.M. Ghazi (1995). Antimicrobial potency of royal jelly collected from queen cells at different larvae ages. Ann. Agric. Sci. Cairo, 40(2): 597-608.

Abdulsalam, K.S. and M.I. Mohamed (1989). Effect of propolis on some Fusarium. Egypt. J. Phytopathol. 21: 149-155.

Ali, M.A.M. (1994). Factors affecting royal jelly production. M.Sc. Thesis, Fac. of Agric. Ain Shams Univ., Cairo, Egypt.

Ali, M.A.M. (2003). Biological and Ecological Studies on Certain Pests of Honey Bee, Ph.D. Thesis, Fac. of Agric. Ain Shams Univ., Cairo, Egypt.

Bailey, L. (1967). The effect of temperature on the pathogenicity of the fungus, *Ascosphaera apis* for larvae of the honey bee, *Apis mellifera*. In insect pathology and microbial control. P.A. van Laan, editor. North Holland Publishing. Amsterdam.

Bamford, S. (1987). Studies on the infection of honey bee larvae with *Ascosphaera apis* Bioliographoc Citation: 200 pp.

Brumfitt, W.; J.M.T. Hamitton-Miller; I. Franklin and J.M.T. Hamillon-Miller (1990). Antibiotic activity of natural products. 1. Propolis. Microbios, 62: 250, 19-22.

Chiu, C.S. and L.K. Chu (1990). Dissipation of oxyletracycline residue in royal jelly. J. Agric. Res. China, 39: 4, 339-346.

Chu, L.K.; T.M. Liu and K.K. Ho (1992). Growth inhibition of *Ascosphaera apis* by royal jelly and 10 hydroxy-2-decenoic acid, Bull Instill. Zool, Acad Sinica, 321: 2, 73-79.

Cochran,w.c. and G.M. Cox(1957). Experiment Design 2nd ed; J . Wilcy and Sons Inc., N.Y-26pp.

El-Dieb, S.M.; M.A. Abd Al-Fattah and A.M. Abd El-Fattah (1997). The antibacterial and antifungal effect of honeybee propolis and its use on Ras, Cheese Bull. Fac. Agric., Cairo Univ., 48: 1, 95-112.

Fujiwara, S.; J. Imai; M. Fujiwara; T. Yaeshima; T. Kawashima and K. Kobayashi (1990). A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. J. Biol. Chem., 265: 19-333-337.

Homitzky, M.A.Z. (1998). The pathogenicity of *Paenibacillus larvae* subsp. Larvae spores and vegetative cells to honey bee (*Apis mellifera*) colonies and their susceptibility to royal jelly. J. Apic. Res. 37: 4, 267-271.

Langoni, H.; P.F. Domingues; S.R.C. Funari; C.G. Chande and I.R. Neves (1996). In vitrjo antimicrobial effect of propolis. Arquivo Brasileiro de-Medicina Veterinana e Zootecnia 1996, 48: 2, 227-229.

Nelson, D.L. and T.A. Gochnauer (1982). Field and Laboratory Studies on Chalkbrood Disease of Honey Bees. Am. Bee J. 122, (1): 29-33.

Okuda, H.; K. Kameda; C. Morimoto; Y. Matsuura; M. Chikaki and M. Jiang (1998). Studies on insulin like substances and inhibitory substances toward angiogenesis converting enzyme in royal jelly, Honey bee Science, 19: 1, 9-14.

Osman, A.R.; M.L. Mohamed and Azza, F. Ashour (1987). Propolis as a possible antiphytopathogenic fungi antibiotic. 5th Cong. Egypt Phytopathol, 506.

Roussy, L. (1962). Nouvell contribution a l etude du *Pericystis apis* (MN) New contribution to the study of *Pericystis apis* (MN). La Gazette Apicole, 63: 101-105.

SAS Institute (1985). SAS/STAT guide for personal computers, version 6 ed SAS Institute, Cary, NC.

Tamura, T.; A. Fujii and N. Kuboyama (1985). Study on mutagenicity of royal jelly. Honeybee Science, 6: 1, 7-12.

Tomoko, S.; S. Kataoka; A. Kataoka; C. Nakajima and T. Hayama (1998). In vitro experimental infection of *Paenibacillus larvae*. Honey bee Science, 19: 4, 145-148.

Xiao, KingWei; Wang, RongJiang; Li ShaoWen; J.W. Xiao; R.J. Wang and S.W. Li (1996). An active peptide inhibiting bacteria in the royal jelly of honey bee. Acta Entomologica Sinica, 39: 2, 133-140.

Yatsunami, K. and T. Echigo (1984). Antibacterial activity of honey and royal jelly. Honeybee Science, 5: 3, 125-130.

تقييم بعض منتجات نحل العسل والمركبات الكيماوية ضد الممرضات الفطرية لحشرة نحل العسل

محمود عبد السميع١ – ناجى يسين عبدالغفار٢

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

٢- قسم أمراض النبات – كلية الزراعة – جامعة عين شمس – شبرا الخيمة القاهرة – مصر

أجريت هذه الدراسة بغرض اختبار وتقييم فعالية بعض منتجات نحل العسل الطبيعية (مثل الغذاء الملكى والبرويوليس وعسل النحل) وبعض المركبات الكيماوية (مثل المونسرين وريزولكس T والتوبسن M) بثلاث تركيزات مختلفة علي نمو الممرضات الفطرية مثل مرض الحضنة الطباشيري المتسبب عن فطر *Ascosphaera apis* ومرض تحجر الحضنة المتسبب عن فطر *Aspergillus flavus*. وأوضحت النتائج المتحصل عليها أن كل منتجات نحل العسل الطبيعية والمركبات الكيماوية خفضت من نمو الممرضات الفطرية المختبرة بالمقارنة مع معاملة المقارنة.وتزداد كفاءة تثبيط الفطريات المختبرة مع زيادة تركيز المواد المختبرة. كذلك وجد أن المركبات الكيماوية كانت أكثر كفاءة في تثبيط الفطريات المختبرة من منتجات نحل العسل. واتضح أن الغذاء الملكى كمنتج طبيعى والتوبسن M كمركب كيماوى كانت أكثر فعالية في التثبيط للفطريات المختبرة في حين أن كلا من البرويوليس أو عسل النحل كمنتجات طبيعية والمونتسرين أو الريزولكس T كمركبات كيماوية كانت أقل فاعلية.