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Article

Synergistic Insecticidal and Biochemical Interactions Between *Brassica napus* Flavonoids and Emamectin Benzoate for Controlling *Spodoptera littoralis*

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Abstract: Despite the development of insect resistance, water contamination and human health hazards, the farmers still depend on excessive application of synthetic chemical insecticides in agricultural practices. Plant-based extracts have been used as bio-pesticides in a wide range due to their toxicity and biodegradability, they complete and enhance the effect of traditional insecticides. They have a wide variety of bioactive secondary metabolite such as flavonoids, a class of polyphenols that have been studied for their role in mechanism of plant defense and their effect as insecticides for crop protection. Phytophagous insects are considered the most destructive pests on crops. The *Brassica napus* L., plant, known as rapeseed or canola, has defensive property against pathogens and phytophagous insects in field. Leaves of *B. napus* are considered as agro-waste, five flavonoids such as *Kaempferol*, *Quercetin-7-O-glucoside*, *Kaempferol-3-O-glucoside and Quercetin*., were isolated and identified from ethanolic *B. napus* leaves extract. This research evaluates insecticidal activity of *B. napus* flavonids, emamectin benzoate and combination of their sub-lethal concentrations on *Spodoptara littorals* and their effect on enzymes activities. All combinations of sub-lethal concentrations of *B. napus* flavonoids and emamectin benzoate increased the mortality percentages of treated larvae, especially, LC_{25} (0.664 g/100ml) of *B. napus* flavonoids with LC_{10} (0.007 mg/L) and with LC_{25} (0.012 mg/L) of emamectin benzoate. The mortality percentages increased by 95.22 and 78.66 Co-toxicity factor, respectively for two combinations. All treatments caused inhibition of phenoloxidase, α-esterase and β- esterases enzymes activities. The greatest inhibition of these enzymes occurred in mixture of LC_{25} of *B. napus* flavonoids with LC_{10} of emamectin benzoate. Phenoloxidase, α-esterase and β- esterases decreased by 60.75, 61.49 and 42.31%, respectively compared with un-treated larvae. The combination of LC_{25} of *B. napus* flavonoids with LC_{25} of emamectin benzoate caused 56.62, 49.50 and 32.11% inhibition in the enzymes activity, respectively. It was concluded that application of *B. napus* flavonoids enhance emamectin benzoate toxicity against *S. littoralis* larvae with less hazard effects on human health, environment and without development of insect resistance.

Key words: Flavonoids, *Brassica napus* L., Emamectin Benzoate, *Spodoptera littoralis*, Synergetic activity.

1. Introduction

One possible way to reduce consumption of synthetic insecticides is by application of natural products derived from plants. Plant-based extracts have been used as bio-pesticides in a wide range due to their toxicity and biodegradability, they complete and enhance the effect of traditional insecticides. They have a wide variety of bioactive secondary metabolite.

Plants synthesize a various bio-active metabolite as terpenoids, steroids, flavonoids, alkaloids, tannins and saponins. Theses metabolites play vital role in plant defense mechanism against phytophgous insects and pathogens **(Kessler and Baldwin, 2002)**.

Flavonoids are polyphenolic compounds found in tissues of plant either in aglycones; free form or glycosides; bonded with sugar molecule. They have fifteen carbon atoms with C_6-C_3-C_6 chain and two benzene rings connected by a linear pyran ring. They can be classified into main classes as flavonols, flavones, flavan-3-ols, flavanones and anthocyanins **(Shen *et al.*, 2022)**. Falvonoids play vital role in plant physiology and biochemistry, they act as detoxification enzymes inhibitors, antioxidants and they have anti-viral, anti-allergic and anti-inflammatory activities **(Winkel, 2001).**

Brassica napus is known as canola or rapeseed. It is a member of *Brassicaceae* family, it has defense secondary metabolites against phytophagous insects **(Chalhoub *et al.*, 2014)**. *B. napus* has a great amount of phenolic compounds, the major metabolites of its leaves and seeds is flavonoids. Its leaves are considered as agro-waste product from the plant. Four flavonoid compounds were isolated from its leaves; *Kaempferol, Quercetin-7-O-glucoside, Kaempferol-3-O-glucoside and Quercetin*. They have insecticidal activity against *S. littoralis* larvae **(Abaza, 2018)**.

So, the efficiency of *B. napus* flavonoids, emamectin benzoate and their mixtures on *S. littoralis* larvae and its effect on some biochemical parameters was studied in this work.

2. Materials and methods

2.1. The Plant

We collect *B. napus* leaves from experimental farm in agricultural research station, Ismailia Governorate. It was cleaned, dried in laboratory temperature and homogenized to fine powder.

2.2. Flavonoids Extraction

The powdered leaves (200 g) was extracted in ethanol 70% *v/v* using Soxhlet apparatus, the extracted solution was subjected to vacuum evaporation using rotary evaporator to yield dried-green residue. The choice of using ethanol in the extraction process depending on the targeted secondary metabolites to be obtained. The concentration of ethanol affected on the flavonoid content, as ethanol concentration increased as extracted flavonoids decreased **(Harmala *et al.*, 1992)**.

2.3. Test insect

The cotton leaf worm, *S. littoralis* strain was cultivated under suitable conditions; 25± 2ºC, 75 ±5% humidity and of 16 hrs light, 8 hrs dark as photoperiod in climatic chamber, larvae were fed on fresh castor bean leaves, the 4^{th} instar larvae were selected for experiments

2.4. Insecticidal Bioassay

Leaf dipping method was applied to estimate the sub-lethal concentrations of the ethanolic plant extract, emamectin benzoate and their combination. The mortality percentages were recorded daily.

The flavonoids-derived extract was formulated in distilled water as emulsions by adding a drop of Tween®-20 (Sigma-Aldrich) as emulsifier to dissolve the material completely in water.

Emamectin benzoate concentrations were prepared as solutions of Speedo®, 5.7% WG (commercial formulation) in distilled water.

The concentrations of *B. napus* flavonoids and emamectin benzoate were prepared serially covering a wide range of mortality, accumulated mortality was recorded daily and corrected **(Abbott, 1925)**. The LC_{10}, LC_{25} and LC_{50} were estimated from the toxicity lines (LC-P lines) of corrected mortality percentage.

For Joint action effect; the sub-lethal concentrations LC_{10}, LC_{25} and LC_{50} of flavonoids-derived extract and emamectin benzoate were combined with each other. The toxicity of applied combinations was evaluated against *S. littoralis* larvae and expressed as Co-toxicity factor. This factor is applied to categorize the obtained data into three distinct categories; synergism or potentiating effect has positive factor (+20 or more), antagonism effect has negative factor (-20 or more) and additive effect has intermediate value of them **(Mansour *et al.*, 1966)**.

2.5. Biochemical bioassay

Aqueous solution of *B. napus* leaves extract and emamectin benzoate at determined LC_{10} and LC_{25} concentrations were prepared separately. Castor leaves were immersed in these solutions and allowed to dry at room temperature. Treated leaves were offered to starved 4th instar larvae for 48 hrs then were fed on fresh untreated leaves. Healthy and survived larvae were collected in clean jar at refrigerator.

Two combination solutions were prepared; *B. napus* flavonoid extract at LC_{25} with emamectin benzoate at LC_{10} and combination of *B. napus* flavonoid extract at LC_{25} with emamectin benzoate at LC_{25}. The bioassay was carried out as the same pattern.

2.5.1. Enzymes characterization

Phenoloxidase enzyme was determined as modification **(Ishaaya, 1971)** Alpa- and Beta-estarases **(Van Asperen, 1962)** for collected healthy and survived larvae.

3. Results and discussion

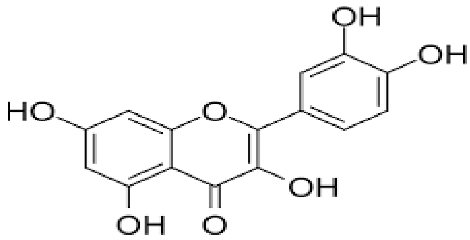
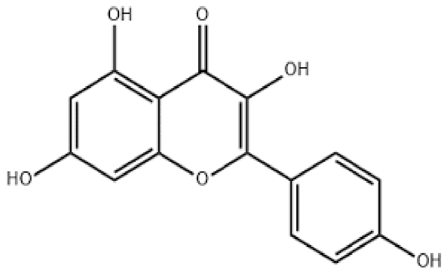
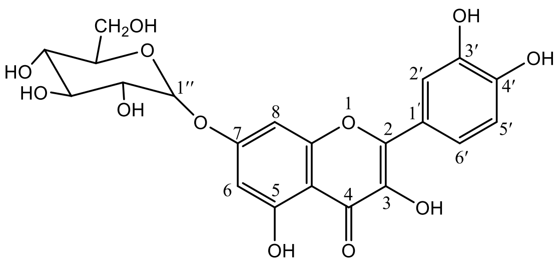
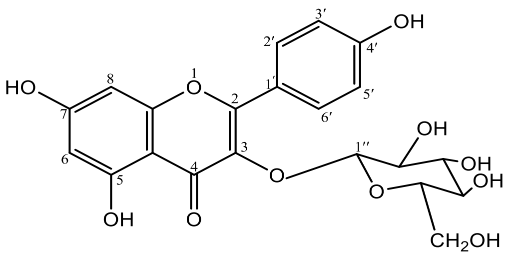
Our study based on *B. napus* plant has defensive property against phytophagous and pathogens in field, it contains secondary metabolites that act as defensive system. Leaves of *B. napus* have no-economic importance, so we select the leaves for this study **(Laoue *et al*., 2022**).

In our previous study some flavonoids as *Kaempferol, Quercetin-7-O-glucoside, Kaempferol-3-O-glucoside* and *Quercetin* were isolated and purified from *B. napus* leaves using Column Chromatography (CC), Thin Layer Chromatography (TLC) and their chemical structures were identified using ^{1}H-NMR and Mass-Spectra **(Abaza, 2018)** as presented in Table (1).

The insecticidal activity of *B. napus* leaves flavonoids, synthetic insecticide (emamectin benzoate) and combination of their sub-lehtal concentrations on *S. littoralis* larvae and their effect on some biochemical parameters were studied.

Emamectin benzoate is a modified chemical compound of avermectin (abamectin). Emamectin benzoate has effective toxicity against lepidopteran insects, especially *S. littoralis* **(Abou-Taleb *et al*, 2009; Davies and Rodger, 2000; Roberts and Hutson, 1999)**.

Table (1). The isolated flavonoids from *B. napus* leaves extract

Compound	Chemical formula
Quercetin	
Kaempferol	
Quercetin-7-O-glucoside	
Kaempferol-3-O-glucoside	

Insecticidal Bioassay

Table (2). Insecticidal activity of *B. napus* flavonoids and emamectin benzoate against *S. littoralis* larvae

Tested compound	LC_{10}	LC_{25}	LC_{50}	Slope ± SE
B. napus flavonoids	0.285 (0.18-0.34) gm/100ml	0.664 (0.153-0.842) gm/100ml	1.833 (0.813-1.936) gm/100ml	0.91±0.24
Emamectin Benzoate	0.007 (0.005-0.008) mg/L	0.012 (0.009- 0.082) mg/L	0.11 (0.081- 0.34) mg/L	1.70 ± 0.08

The sub-lethal concentrations LC_{10}, LC_{25} and LC_{50} were determined as insecticidal parameters of evaluated compounds. The percentages of mortality are directly proportional to the applied concentrations and time post treatment as shown in Table (2).

Table (3). combination of *B. napus* flavonoids and emamectin benzoate against *S. littoralis* larvae

Compound	Expected mortality		Observed mortality		Co-toxicity factor	
B. napus flavonoids	LC_{10}	LC_{25}	LC_{10}	LC_{25}	LC_{10}	LC_{25}
Emamectin benzoate						
LC_{10}	20	35	28.67	68.33	43.35	95.22
LC_{25}	35	50	46.67	89.33	33.34	78.66

Using of insecticide mixtures is recommended in agricultural practices to improve the pest controlling and to delay the insect resistance for insecticides. The obtained results in Table (3) showed all combinations of *B. napus* flavonoids and emamectin benzoate at sub-lethal concentrations increased the mortality percentages of treated larvae especially, LC_{25} (0.664 gm/100ml) of *B. napus* flavonoids with both of LC_{10} (0.007 mg/ L) and LC_{25} (0.012 mg/L) of emamectin benzoate. The observed mortality increased with 95.22 and 78.66 Co-toxicity factor, respectively. The flavonoids of *B. napus* extract synergist the emamectin benzoate activity. Therefore, the mixture of *B. napus* leaves extract and emamectin benzoate will reduce the concentration and number of applications of emamectin benzoate in field.

Table (4). Biochemical parameters of *S. littoralis* larvae treated with sub-lethal concentrations of *B. napus* flavonoids, emamectin benzoate and their combination

Treatment	Enzyme activity± SE					
	Phenoloxidas O.D. unit *x*10^3/min./g. b. wt		α-esterase µg α-naphthol/min./mg protein		β-esterase µg β-naphthol/min./mg protein	
	activity	Inhibition %	activity	Inhibitio n%	activity	Inhibition %
B. napus flavonoids LC_{25}	10.21± 0.8	46.064	11.26±0.13	44.69	21.2 ±0.46	33.79
emamectin benzoate LC_{10}	12.01± 0.5	34.89	18.01±0.23	11.54	28. 94±0.36	9.61
emamectin benzoate LC_{25}	14.84± 0.82	21.60	17.29 ± 0.18	15.07	28.58 ±0.7	10.74
B. napus flavonoids LC_{25} + emamectin benzoate LC_{10}	7.43± 0.16	60.75	7.84 ± 0.06	61.49	18.47± 0.23	42.31
B. napus flavonoids LC_{25} + emamectin benzoate LC_{25}	8.21± 0.21	56.62	10.28 ± 0.09	49.50	24.62± 1.72	32.11
Control	18.93±1.61	--	20.36 ±0.24	--	32. 02 ± 0.6	--

By measuring the activities of some enzymes in treated larvae; all treatments caused inhibition of phenoloxidase, α-esterase and β- esterases activities. The greatest inhibition in phenoloxidase, α-esterase and β- esterases occurred in the combination of LC_{25} of *B. napus* flavonoids with LC_{10} of emamectin benzoate; they decreased by 60.75, 61.49 and 42.31%, respectively compared with un-treated larvae.

Phenoloxidase, α-esterase and β- esterase enzymes activities decreased for the treatment with the combination of LC_{25} of *B. napus* flavonoids with LC_{25} of emamectin benzoate, the inhibition percentages are 56.62, 49.50 and 32.11%, respectively compared with un-treated ones. These results are compatible with results of toxicity evaluation of tested compounds and their mixture, where the mixture of sub-lethal concentrations of *B. napus* leaves extract and emamectin benzoate achieved the most toxic effect on the treated larvae. Emamectin benzoate recorded high toxicity towards many of lepidoptera species, with LC_{90} varying from 0.0050 to 0.0218 µg/ml **(Argentine *et al*., 2002).**

4. Conclusion

This research indicates that combination of sub-lethal concentration of *B. napus* flavonoids with sub-lethal concentrations of emamectin benzoate, as synthetic insecticide enhances the toxicity of *S. littoralis* larvae. These combinations significantly inhibited phenoloxidase, α-esterase, and β-esterase enzymes activities. Therefore, application of this mixture in field proves to be an effective strategy for pest management, diminishing insect resistance to synthetic insecticides while also reduce potential hazards to the environment and human health.

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