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## Schiff base synthesis, formulation as emulsifiable concentrate and study its nematicidal efficiency on root-knot nematode *Meloidogyne* spp. under greenhouse conditions

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Hamouda, S. E. S., Abd-Alla, H. I. and Abd Elattif, Nosa S. (2022). Schiff base synthesis, formulation as emulsifiable concentrate and study its nematicidal efficiency on root-knot nematode *Meloidogyne* spp. under greenhouse conditions. International Journal of Agricultural Technology 18(5):1983-1994.

**Abstract** The reaction of primary amines with carbonyl compounds has been recognized as a source of Schiff bases. The biological activity of new imines (Schiff bases) against *Meloidogyne incognita* was investigated. According to the bioassay, all investigated chemicals had promised as nematicidal effectiveness. Standard approach was used to synthesize Schiff base from salicylic aldehyde (2-hydroxybenzaldehyde) and anthranilic acid (2-aminobenzoic acid). Spectral analysis revealed its chemical structure. The Schiff base physio-chemical characteristics were investigated. The results revealed that emulsifiable concentrate (EC) was the best formulation form. It was developed as 10% emulsifiable concentrate. The new formula's physio-chemical properties were investigated. It was tested against root-knot nematode, *Meloidogyne* spp. in the second-stage larvae under greenhouse conditions. The new formula significantly decreased the amount of galls/root, eggs, and limited the larvae's ability to penetrate eggplant roots, with a direct proportion between concentration and effect in both cases. The novel formulation showed 38.4, 92.8, 99.3, and 100 percent gall reduction and 52.6, 94.7, 100, and 100 percent egg reduction at 10, 100, 1000, and 10000 ppm, respectively. The new EC formulation might be used to control the root-knot nematode, *Meloidogyne* spp.

**Keywords:** Emulsifiable Concentrate, Root-Knot Nematode and Schiff Base

### Introduction

Plant parasitic nematodes (PPNs) have been identified as a serious threat to world food security, with almost 4100 species recorded to date (Nicol *et al.*, 2011). The huge phylum nematoda, which includes un-segmented roundworms, includes nematodes. Nematodes are cosmopolitan in nature, occurring in nearly every ecosystem on the planet. They can adapt to a wide range of climates, from cold to hot in deserts. Plant parasitic nematodes are classified as ectoparasites or endoparasites based on their feeding patterns and lifestyles.

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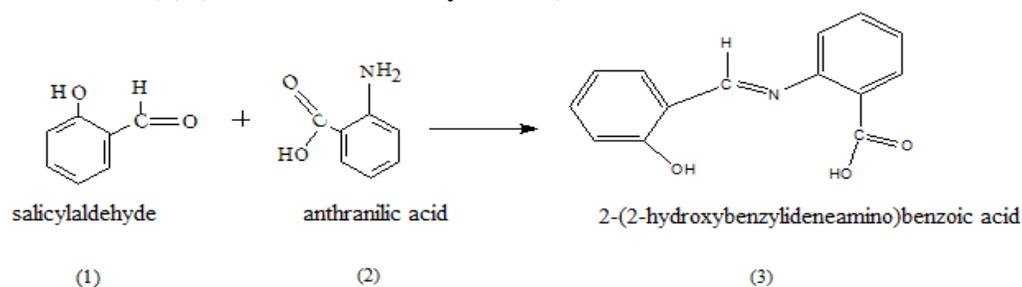
Ectoparasites feed by putting the stylet into root cells that are outside on the root surface, whereas endoparasites feed by piercing host cells and feeding from within (as reviewed by Escobar *et al.*, 2015). *Meloidogyne* is a genus with roughly 98 species that may parasitize a wide variety of vascular plants (Moens *et al.*, 2009). To infiltrate the host, second-stage juveniles use both physical and enzymatic methods. They break the plant cell wall with a stylet and then release cellulolytic and pectolytic enzymes to totally digest it. Root-knot nematodes are the most important diseases, attacking a wide range of host plants and inflicting significant economic losses, particularly in vegetable crops (Mitkowski and Abawi, 2003). Root-knot nematodes, *Meloidogyne* spp., are widely scattered throughout Egypt, causing significant agricultural losses of 30-40% of yields (El-Deeb *et al.*, 2018). The root-knot nematode attacks vegetables, causing root galling and vascular damage that disrupts water and mineral intake, resulting in a significant production decline (Abolusoro *et al.*, 2013). Plant-parasitic nematodes are thought to be responsible for roughly 100 billion \$ in agricultural losses (Chitwood, 2003). Despite the fact that many nematode species exhibit physiological specialization in respect of food or host ranges, as well as temperature, no nematode species has developed resistance to nematicides. Only one record was reported by Castro and Thomason (1971), although it was inconclusive. They were able to obtain a population of *Aphelenchus avenae* that was resistant to ethylene dibromide (EDB). Furthermore, the Insecticide Resistance Action Committee observed that while there is good evidence that nematicide resistance can occur in the lab, it is less apparent whether it can occur in the field. While there is excellent evidence that nematicide resistance can occur in the lab, it is less clear whether it can occur in the field, according to the Insecticide Resistance Action Committee. The resistance of field populations to nematicides has not been widely studied, and it is surprised to be low in comparison to the levels of resistance seen in mammalian parasites. "Resistance of nematodes to soil fumigants has yet to be reported, but systemic nematicides are relatively new, and it is probably only a matter of time until resistance does arise," according to a recent National Academy of Sciences monograph (Berenbaum, 2000). The free-living nematodes *Rhabditis oxycerca* was developed for 400 generations to produce strains that could reproduce at 600 and 480 g/ml aldicarb and oxamyl concentrations, respectively. The two mutant strains differed from wild type in terms of size (especially in the tail area), tolerance of warm temperatures, offspring production, and movement in electric fields, among other traits. The wild type showed decreased motility, electric field migration, and reproduction in nematicide solutions (Kampfe and Schütze, 1995). In another study, researchers found that genetically modified strains of the insect pathogen

*Heterorhabditis bacteriophora* were 8-70 times more resistant to fenamiphos, avermectin, and oxamyl (Glazer *et al.*, 1997). In the absence of further nematicide pressure, the improved resistance remained persistent; the strains have evident potential utility in integrated pest management systems.

Since 1864, when Hugo Schiff described the condensation of primary amines with carbonyl compounds, Schiff bases have been identified (Cimerman *et al.*, 2000). The biological activity of novel imines (Schiff bases) against *Meloidogyne incognita* was examined (Kundua *et al.*, 2009). All of the substances examined displayed promising nematicidal action, according to the bioassay. In addition, antimicrobial characteristics can also be found in Schiff bases also including 2-aminothiophenol, 2-aminophenol, 2-aminobenzoic acid, and 2-amino 3-hydroxypyridine (Raman *et al.*, 2011).

A pesticide formulation is a blend of active and inert chemicals that results in a pesticide that is suitable to use. Pesticides are being developed to make them safer and more convenient to use. This is due to the fact that many pesticide active constituents are not suitable for usage in their "pure" (technical grade) form. Others are extremely harmful when concentrated, many do not mix well with water, some are unstable, and some are difficult (or hazardous) to handle, move, or store. To address these difficulties, manufacturers add inert components to end-use pesticide products. Some inert substances are only diluents or carriers with no pesticidal properties. In many cases, these inert substances improve the finished products safety, ease of use, and effectiveness (Pesticide formulation, 2017). In light of this, the synthesis of Schiff base from the condensation of salicylic aldehyde (2-hydroxybenzaldehyde) and anthranilic acid (2-aminobenzoic acid) reported herein. Spectroscopic investigations were used to identify the Schiff base (IR, Mass spectrometry and  $^1\text{H-NMR}$ ). Its nematicidal action against root-knot nematode was investigated in a greenhouse setting, and it was prepared as an emulsifiable concentrate as a first step in nematode control.

Salicylic aldehyde (2-hydroxybenzaldehyde) (1) and anthranilic acid (2-aminobenzoic acid) (2) were used according to standard procedure to produce Schiff base (3) (Xavier and Srividhya, 2014).



In a round bottomed flask equipped with a magnetic stirrer, an ethanolic solution of salicylic aldehyde (1) (1 mmol, 0.122 ml) was mixed with anthranilic acid (2) (1 mmol, 0.137 g). The reaction mixture was simmered for 10 hours under reflux. On a water bath, the resultant solution was concentrated to 8 mL and allowed to cool to 0 °C. The produced crystalline red precipitate was filtered out, washed with ethanol several times, and dried under vacuum over CaCl<sub>2</sub> Red crystals; yield 90 %; (ethanol); melting point 212 °C; IR (potassium bromide) v/cm<sup>-1</sup> 3436 (COOH); 3270 (OH); 1690 (CO); 1622 (CN), proton-NMR (300 MHz, DMSO-d<sub>6</sub>): δ/ ppm = δ: 6.47-6.94 (s, 1H, HC=N), 7.01-8.86 (m, Ar-H), 10.26 (s, 1H, OH) and 11.22 (s, 1H, COOH), MS m/z (%) = 241 (29), 224 (50), 196 (91), 120 (41), 148 (70) and 93 (73).

The major goal of this study is to use a newly formulated Schiff base as an emulsifiable concentrate (EC) as nematicide to control root-knot nematode *Meloidogyne* spp.

## Materials and Methods

### *The used chemicals*

Chemicals for synthesis was used as salicylic aldehyde (2-hydroxybenzaldehyde), 122.12 g/mol and anthranilic acid (2-amino benzoic acid), 137.14 g/mol were purchased from Al Obour Pharmaceutical Industrial Co., Cairo, Egypt. Surface active agents were Tween 80, Toximol and poly ethylene glycol 600 dioleate (PEG 600 dioleate) were supplied by AL-gomhoria Company, Cairo, Egypt. Solvents were acetone, Xylene, absolute ethanol and DMF (di methyl formamide) were supplied by AL-gomhoria Company, Cairo, Egypt.

### *Physico-chemical properties of formulation basic constituents*

#### **Active ingredient**

Solubility was done by the volume of distilled water, xylene, acetone, and DMF which used to determine one gram of active component's total solubility or miscibility at twenty °C to measure according to (Nelson and Fiero, 1954). The percent solubility was determined using the following formula;

$$\% \text{ solubility} = W/V \times 100 \dots\dots\dots (1)$$

Where W is the active component weight and V is solvent volume necessary for full solubility.

b) Free acidity or alkalinity: It was determined in accordance with WHO guidelines (1979).

### ***Surfactants***

Solubility was calculated as mentioned before. Hydrophilic-lipophilic balance (HLB) was done by surfactant solubility in water which is taken as an early measure of the hydrophilic-lipophilic balance (HLB) of the surfactant (Lynch and Griffin, 1974). Free alkalinity or acidity was calculated as mentioned before. Critical micelle concentration (CMC) was done by the concentration at which a solutions surface tension did not show any change when the surface active agent concentration increases. CMC of the surfactants were tested and calculated using the method of Osipow (1964). Surface tension was done using the solution containing 0.5 percent (Weight/Volume) surfactant and measured using a Du-Nouy tensiometer according to ASTM D-1331 (2001).

### ***Local prepared emulsifiable concentrate (EC) formulation***

For active ingredient that does not dissolve in water, emulsifiable concentrate (EC) is the best choice. The Schiff base was prepared as an emulsifiable concentrate according to (Soliman, 2005). A precise amount of Schiff base was taken, dissolved in enough solvent, and then an emulsifier was added, followed by one hour of stirring. To make it 10 ml, the solution was transferred to a measuring flask and topped up with the same solvent. To homogenize the solution, the flask was shaken and the following physico-chemical characteristics of the formulation were determined. Emulsion stability test was performed in accordance with FAO/WHO MT 36.3 (2010). Accelerated storage was based on the method of (Dobrat and Martijn, 1995). Free alkalinity or acidity: It was calculated according to (Dobrat and Martijn, 1995).

Spray solution with field recommended dilution rate was performed by the surface tension was calculated as stated before. pH was measured as a Cole-Parmer pH conductivity meter 1484-44 was used to determine it (Dobrat and Martijn, 1995). Viscosity was examined using Brookfield viscometer model DVII+Pro to determine with cm poise as measurement unit based on ASTM D-2196 (2005). Electrical conductivity was measured with a Cole-Parmer pH/Conductivity meter 1484-44, where  $\mu$  mhos are the unit of measurement (Dobrat and Martijn, 1995).

### ***Bioassay***

In glass vials containing root-knot nematode *Meloidogyne* spp. of 1000 2<sup>nd</sup> stage larvae, serial concentrations from the 10% emulsifiable concentrate

were prepared at 10, 100, 1000, and 10000 ppm and stored for 24 hours under laboratory conditions. Then, three times each treatment was replicated, each concentration was transferred to an eggplant cup through holes around each plant. Three cups were left untreated as control. The cups were irrigated on a regular basis as required. The number of galls per root was calculated to determine the nematode 2<sup>nd</sup> stage larvae capability to penetrate eggplant roots. The results were compared to the control after seven weeks of treatment (Ibrahim *et al.*, 2014).

## Results

### *Schiff base as active ingredient physico-chemical properties*

Result showed that Schiff base had shown incomplete solubility in both aqueous and organic solvents (water, acetone, and xylene), with the exception of DMF, it showed weak solubility (5.9 percent). It was shown to have a very weak alkaline property based on free alkalinity. Schiff base had a melting point of 210-212 °C as shown in Table 1.

**Table 1.** Schiff base (3) as active ingredient physico-chemical properties

Solubility % (W / V)				Free alkalinity as NaOH	Melting point °C
Water	Acetone	Xylene	DMF		210-212
-*	-	-	5.9	0.06	

-\* : means insoluble.

### *Physico-chemical properties of surface active agents*

The physico-chemical parameters of the suggested surface active agents for formulating Schiff base were shown in Table 2. Three different surface active agents were investigated (Toximole, Tween 80 and polyethylene glycol 600 dioleate). Toximole and Tween 80 had weak acidic property (measured as sulfuric acid), whereas PEG 600 dioleate had a slight alkaline property (measured as sodium hydroxide). Furthermore, the HLB values of all chosen surfactants range from (10-14), indicating the type of surfactant and its role in the formulation process. Moreover, the three surface active agents displayed comparatively low values of solution surface tension.

**Table 2.** Suggested surface active agents phsico-chemical properties

Surface active agents	Surface tension dyne/cm	CMC	HLB	Acidity as H <sub>2</sub> SO <sub>4</sub>	Alkalinity NaOH	as
Tween 80	48	0.5	13-14	0.61	-	
P.E.G 600 Do*	35.8	0.9	10-12	-	0.25	
Toximol	34.99	0.9	10-12	0.03	-	

P.E.G 600 Do\*: polyethylene glycol 600 dioleate.

***Physico-chemical parameters of local 10% emulsifiable concentrate formulation stored under various conditions***

The physico-chemical features of the locally manufactured (EC) formulation were shown in Table 3 under the recommended storage conditions for emulsifiable concentrates. Under normal or accelerated storage conditions, the produced formulation passed emulsion stability and spontaneity tests well; no oil separation, precipitation, or cream separation were observed. When stored under normal or hot conditions, the new formula exhibited nearly similar values of free acidity as sulfuric acid. Before and after accelerated storage, there were no changes observed in the physico-chemical properties of the newly prepared local (EC) formulation.

**Table 3.** Schiff base local formulation physico-chemical properties under normal and accelerated storage conditions

Before storage					Cold storage	After storage				
Suspensibility		Emulsion stability		Free acidity as H <sub>2</sub> SO <sub>4</sub>		Suspensibility		Emulsion stability		Free acidity as H <sub>2</sub> SO <sub>4</sub>
Hard	Soft	Hard	Soft			Hard	Soft	Hard	Soft	
100 %	100%	pass	pass	0.21	pass	100	100%	pass	pass	0.22
						%				

Physico-chemical characteristics of spray solution at recommended field dilution rate of 0.5 percent was done. Result showed that the spray solution of the locally produced formulation (0.5 percent) had low surface tension (39.5 dyne/cm), high viscosity (9.8 cm poise), high electrical conductivity (100 μ mhos), and a low PH value (5.45) at field dilution rate Table 4.

**Table 4.** Spray solution physical properties by using field dilution rate 0.5%

<b>PH</b>	<b>Conductivity µmhos</b>	<b>Viscosity poise</b>	<b>cm</b>	<b>Surface tension dyne/cm</b>
5.45	100	9.8		39.5

The new EC formulation biological activity showed the ability of root-knot nematodes *Meloidogyne* spp. 2<sup>nd</sup> stage larvae to penetrate eggplant roots under greenhouse conditions.

The Schiff base 10% (EC) formulation with 10, 100, 1000, and 10000 ppm reduced the number of galls per root by 91.7, 9.7, 1, and 0.3, respectively, as shown in Table 5. The same concentrations reduced the number of eggs per root to 9, 1, 0 and 0 respectively under greenhouse conditions.

**Table 5.** Effect of the new local prepared 10 % emulsifiable concentrate formulation on penetration of eggplant roots by root-knot nematode 2<sup>nd</sup> stage larvae under greenhouse conditions

<b>Concentration (ppm)</b>	<b>Number of galls</b>	<b>% of gall reduction</b>	<b>Gall index</b>	<b>Number of eggs</b>	<b>% egg of reduction</b>
Control	134.3	0	5	19	0
10	91.7	38.4	4	9	52.6
100	9.7	92.8	2	1	94.7
1000	1	99.3	1	0	100
10000	0.3	100	1	0	100

\*: Gall index 0 to 5. Where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = + 100 (root system completely galled) (Taylor and Sasser, 1978).

## Discussion

The required physico-chemical parameters of compound were elucidated in order to find the best formulation type for preparing this substance. Several aspects must be considered while choosing a pesticide formulation. These considerations include the risks and benefits of numerous choices, the practicality of using a certain formulation in a specific place to manage the target pest, and whether the manufactured product will provide effective control (Fishel, 2010). Schiff base studied physico-chemical properties, determined the most suitable formulation was emulsifiable concentrate (EC). The major attribute that guides the formulation processes to emulsifiable concentrates is the Schiff base's insolubility in aqueous solvent and weak solubility in dimethyl formamide (Abd-Alla and Hamouda, 2021). In addition, surface active agents physico-chemical properties demonstrated the compatibility in properties either acidity or alkalinity, HLB, CMC and surface tension with active ingredient, which was one of the most important determinants of the success of the new

formulation. The Schiff base as an active ingredient showed free alkalinity, and one of the proposed surfactants (P.E.G 600 Do), also it showed free alkalinity, indicating that it might be employed in the formulation procedure of this chemical with no chemical interaction expected (Libs and Salim, 2017).

Many trials were conducted in order to formulate Schiff base as an emulsifiable concentrate (EC). The physico-chemical properties of the locally manufactured (EC) formulation demonstrated no oil separation, precipitation, or cream separation during normal and accelerated storage conditions. The physico-chemical parameters of the manufactured local (EC) formulation showed no differences before and after accelerated storage, indicating that the formulation may maintain its properties under different storage conditions with expected stability (Hala *et al.*, 2016).

When using the recommended field dilution rate, the spray solution had low surface tension, high viscosity, high conductivity, and a low PH value. A decrease in the surface tension of a pesticide spray solution predicts enhanced wettability and spreading on the treated surface, resulting in increased pesticidal efficiency, according to Pereira *et al.* (2016). Increasing viscosity of the spray solution had lower drift while enhanced retention and insecticidal efficacy (Spanoghe *et al.*, 2007). According to El-Sisi *et al.* (2011), deionization of pesticides and greater deposit and penetration in the tested surface were resulted to increase the electrical conductivity and decreased pH (acidic) values in insecticidal spray solution, resulting in an increase in insecticidal efficacy. The Schiff base 10 % (EC) formulation inhibited the ability of penetration of the root-knot nematode *Meloidogyne* spp. 2<sup>nd</sup> stage larvae which resulted in the reduction of the number of galls/root. An inverse proportionating relationship was noticed, as the concentration of the new formula increased, the number of galls/root decreased in addition to the number of eggs which also decreased with the gradual increased in the concentration of the new formulation (Adaji *et al.*, 2021). In addition to the action of active ingredient to control the target pest, surface active agents (surfactants) play a vital role in achieving this control on inhibition. The terms adjuvant and surfactant are often used interchangeably. Because all surfactants are adjuvants, these words might refer to the same product. Adjuvants are compounds that have no pesticidal properties. To improve mixing, application, or pesticidal effectiveness, adjuvants are either pre-mixed in the pesticide formulation or added to the spray tank (Libs and Salim, 2017).

It concluded that the schiff base was synthesized and formulated as a 10% emulsifiable concentrate. The new formula passed all emulsifiable concentrates tests, as well as testing on the number of galls/root as an indicator of the ability of root-knot nematode *Meloidogyne* spp. 2<sup>nd</sup> stage larvae to

penetrate eggplant roots and the number of eggs under greenhouse conditions. The amount of galls/root, eggs, and the potential of 2<sup>nd</sup> stage larvae to penetrate eggplant roots were dramatically reduced. The novel formula might be used in the field to control root-knot nematodes.

## Acknowledgements

The authors would like to extend their sincere thanks and appreciation to their professor who taught and supervised them, Prof. Dr. Ashraf Mahmoud Abdel Basset Al-Qadi and pray for his long life. The authors are also thankful to the technical team of Formulation Research Department, Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Dokki, Giza, Egypt for their technical assistance.

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(Received: 18 December 2021, accepted: 30 July 2022)