Botanical nematicides, Prospects for Development and Implementation.

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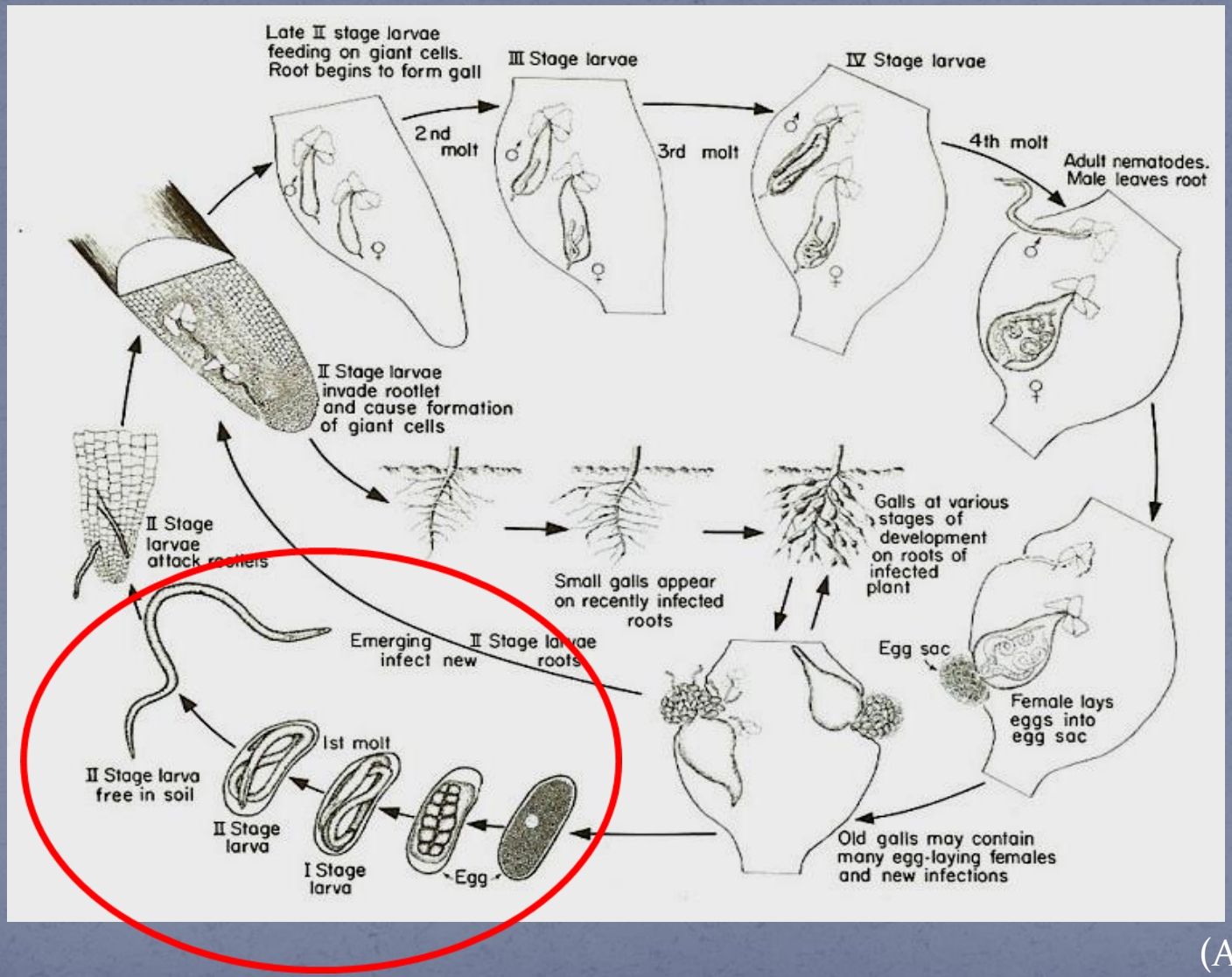
The root knot nematodes *Meloidogyne* sp.

Economically important pest

(Chitwood, 1949; Trudgil and Blok, 2001; Back et al., 2002

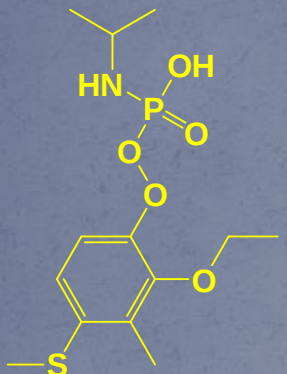
Biological cycle *Meloidogyne* sp.



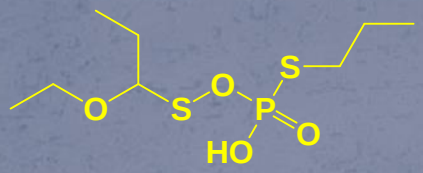
(Agrios, 1997)

Synthetic nematicides

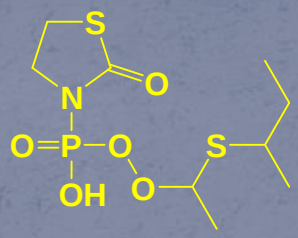
Montreal Protocol- 91/414/ECC- 2009/128/EC 1107/2009



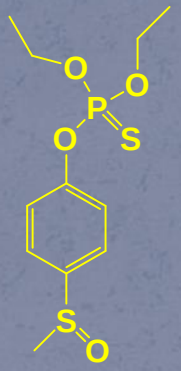
fenamiphos



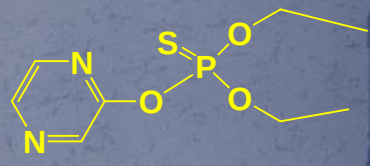
ethoprophos



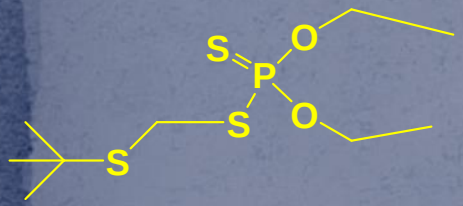
fosthiazate



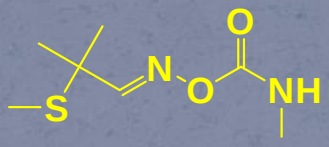
fensulfothion



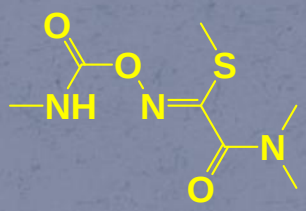
thionazin



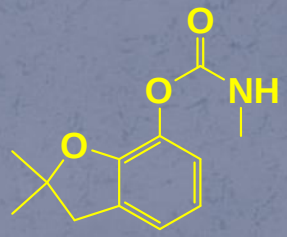
terbufos



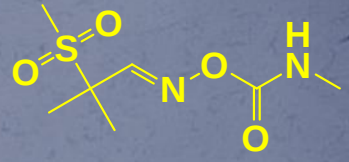
aldicarb



oxamyl

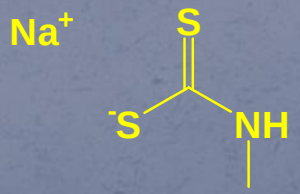


carbofuran

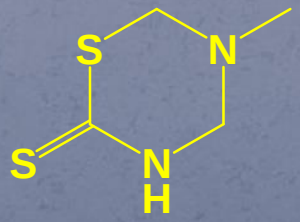


aldoxycarb

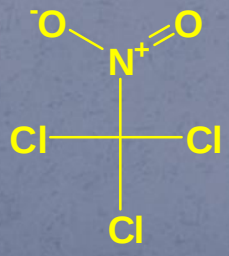
1.3 dichloropropene



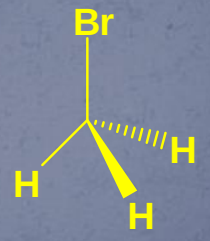
metam-sodium



dazomet

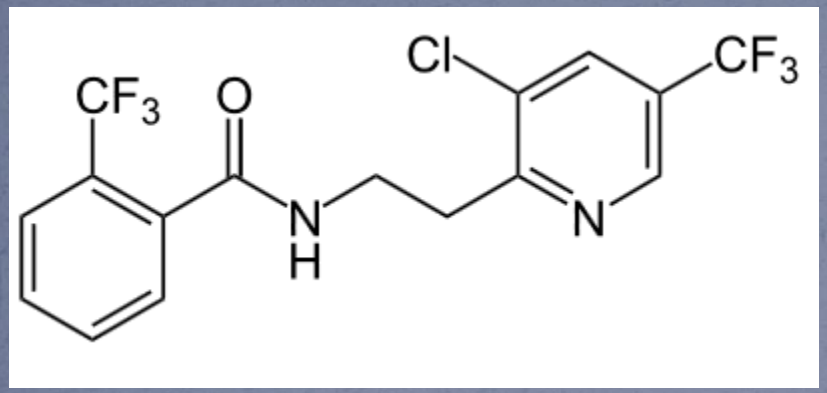


chloropicrin

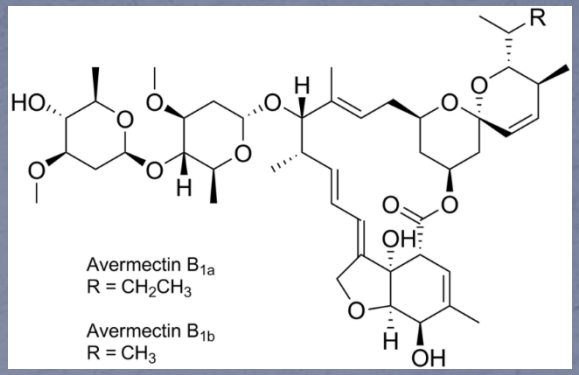


methyl bromide

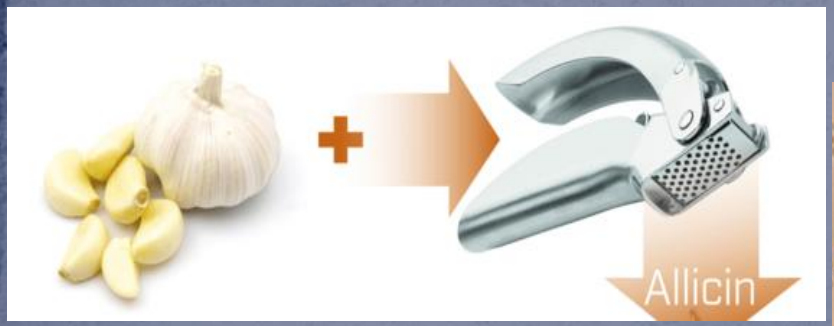
New nematicides



fluopyram (2016)

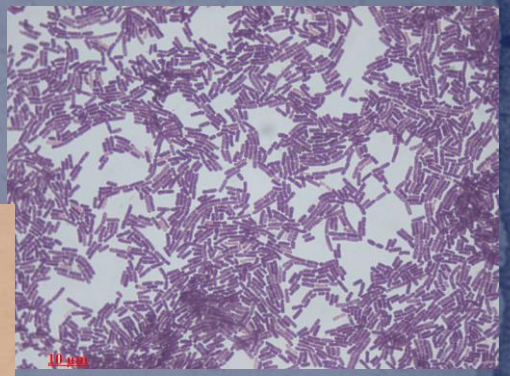


abamectin (2017)



garlic extract (2015)

Paecilomyces lilacinus (2017)



Bacillus firmus (2014)

Everything the plants have that you want.

Botanical nematicides

Advantages

- Easy to apply under field conditions (soil amendments)
- Easy, cost effective and safe to produce (aqueous extracts)
- Resistance management – complex mixtures of compounds with different modes of action
- Secondary plant enhancement properties
- Small half life and low residues
- Safe to natural enemies, beneficials and higher organisms
- Reduction of risks and adverse impacts from pesticide use for human health and the environment.
- Growing market preference

Botanical nematicides

Restrictions

- Seasonal availability of raw material
- Variability in chemical composition and subsequent biological activity
- Instability under field conditions & storage
- **Strict legislation considering authorization**

Botanical nematicidals - application types

A. Soil amending with botanical material

B. Drip irrigation with aqueous botanical extracts

C. Development of new nematicides based on plant secondary metabolites – a.i.

A. Botanical materials (Soil amending)

1. Crop rotation and incorporation
2. Recycling of solid agricultural wastes
3. Recycling of pruning wastes
4. Weeds incorporation

Crop rotation & incorporation : *Petroselinum crispum* – parsley

- Soil amending with fresh plant parts reduces *Meloidogyne incognita* ♀ counts in host root, and the **EC50 value is calculated at 24.7 mg/g soil**

- A dose response is manifested between the botanical mass incorporated into the soil and the host roots' and aerial part weight, at the dose range of 4 to 100 mg/g soil.

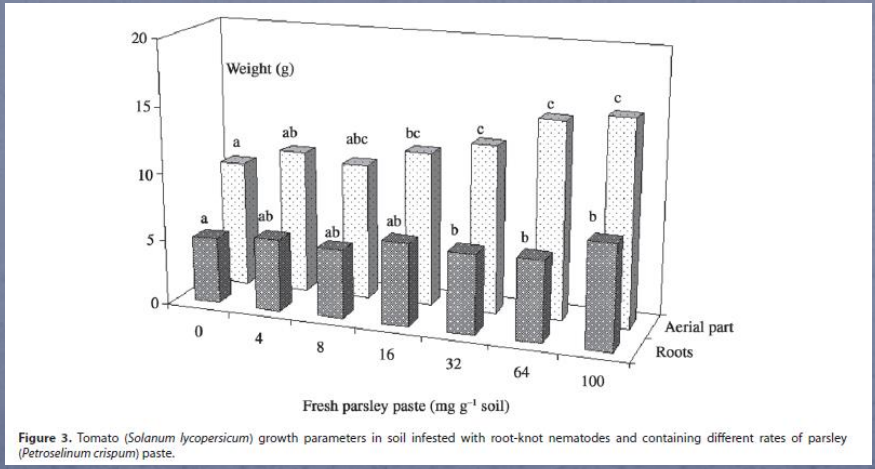


Figure 3. Tomato (*Solanum lycopersicum*) growth parameters in soil infested with root-knot nematodes and containing different rates of parsley (*Petroselinum crispum*) paste.

Ntalli et al., Pest Manag. Sci 2015, 71(8):1099-105.

Crop rotation & incorporation: *Eruca sativa* – rucola

- Soil amending with fresh plant parts reduces *Meloidogyne incognita* ♀ counts in host root, and the **EC_{50} value is calculated at 20 mg/g**

- A dose response is manifested between the botanical mass incorporated into the soil and the host roots' and aerial part weight, at the dose range of 4 to 128 mg/g soil.

Aissani et al., JAFC 2015, 63:6120-5

Recycling of juicing wastes: *Citrus cinensis* – orange peel

- Soil amending with fresh plant parts reduces *Meloidogyne incognita* & *Meloidogyne javanica* ♀ counts in host root, and the **EC50 value is calculated at 8,7 & 2 mg/g soil**
- Phytotoxicity at 100 mg/g soil.

Ntalli et al., J Arg. Sci .Tech. 2013, 3, 603-616

Recycling solid aromatherapy wastes:

Rosemary – (Rosmarinus officinalis)

- Soil amending with dry plant parts reduces *Meloidogyne incognita* & *Meloidogyne javanica* ♀ counts in host root, and the EC50 value is calculated at **EC_{50} = 2.8 & 5.7 mg/g soil**

Levander – (Lavandula angustifolia)

- Soil amending with dry plant parts reduces *Meloidogyne javanica* ♀ counts in host root, and the EC50 value is calculated at **EC50 = 3 mg/g soil**

Ntalli et al., J Arg. Sci .Tech. 2013, 3, 603-616

Recycling of pruning wastes: Chinaberry – *(Melia azedarach)*

- Soil amending with fresh ripe fruits reduces *Meloidogyne incognita* counts in host root, and the EC50 value is calculated at **EC_{50} = 4 mg/g soil**

- A dose response is manifested between the botanical mass incorporated into the soil and the host roots' and aerial part weight, at the dose range of 0,05 to 6,4 mg/g soil.

- Plant enhancement properties

Ntalli et al., Ananls Appl. Biol 156, 309-317; Cavoski et al., 2012 Crop Prot. 35, 85-90

Recycling of pruning wastes: Tree of heaven – *(Ailanthus altissima)*

- Soil amending with chopped fresh wood reduces *Meloidogyne incognita* counts in host root, and the EC50 value is calculated at **EC50 = 2 mg/g soil**
- A dose response is manifested between the botanical mass incorporated into the soil and the host roots' and aerial part weight, at the dose range of 1 to8 mg/g soil.
- Phytotoxicity at 16 mg/g soil.

Ntalli et al., J Pest Sci 2016, 89, 565-579

Weeds incorporation:

Nightshade *(Solanum nigrum)*

- Soil amending with fresh ripe fruits reduces *Meloidogyne incognita* counts in host root, and the EC50 value is calculated at **EC50 = 1 mg/g soil**

Jimsonweed *(Datura stramonium)*

- Soil amending with fresh ripe fruits reduces *Meloidogyne incognita* counts in host root, and the EC50 value is calculated at **EC50 = 11 mg/g soil**

Ntalli et al., J Nem (DOI: 10.21307/jofnem-2018-017)

B. Aqueous botanical extracts (Drip irrigation)

Aqueous botanical extracts

Mint

- Efficacy of 3 mint species (*M. piperita*, *M. spicata*, *M. pulegum*) on the paralysis of *M. incognita* after 72 h of immersion:
  aqueous extracts EC_{50} = 1000, 300 & 745 µg/mL
  methanolic extract : $EC_{50/72h}$ > 1000 µg/mL
  essential oil : EC_{50} > 1000, 358 & >1500 µg/mL

Chinaberry (Melia azedarach)

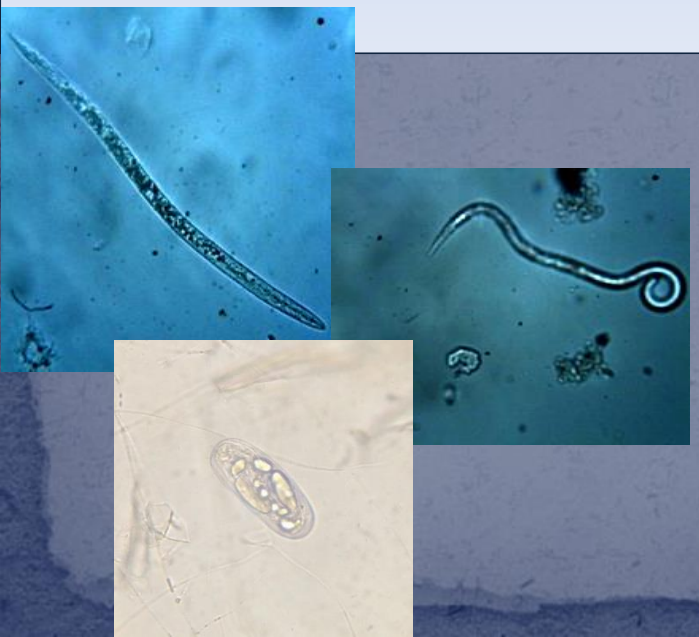
- Efficacy of aqueous extracts of ripe fruits of *M. azedarach* on the paralysis of *Meloidogyne incognita* after immersion for 72 h:

EC_{50} = 400 µg/mL

Caboni et al., JAFC 2013, 61: 9784-9788; Cavoski et al., Crop Prot. 2012, 35: 85-90

C. Plant Secondary Metabolites – a.i

- Essential oils
- Methanolic extracts
- Single compounds and binary mixtures



Essential oils

1. *Melissa officinalis*
2. *Sideritis clandestina*
3. *Origanum dictamnus*
4. *Ocimum basilicum*
5. *Mentha pulegium*
6. *Origanum vulgare*
7. *Vitex agnus castus*
8. *Salvia officinalis*
9. *Eucalyptus meliodora*
10. *Laurus nobilis*

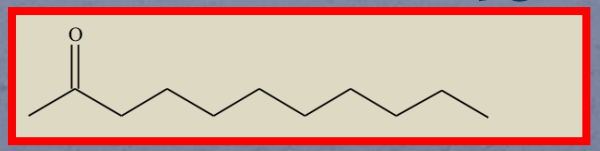
11. *Pistacia terebinthus*
12. *Foeniculum vulgare*
13. *Pistacia anisum*
14. *Achillea millefolium*
15. *Citrus cinensis*
16. *Lavandula stoechas*
17. *Rosmarinus officinalis*
18. *Juglans regia*
19. *Ruta chalepensis*

Essential oils - ketones

Plant species	*J2s paralysis bioassays* $EC_{50/1d}$ (µg/ml)	R^2
R. chalepensis	80	0.98
F. vulgare	110	0.92
P. anicum	120	0.98
E. meliodora	830	0.95
I. viscosa	910	0.99
P. terebinthus	1040	0.89
O. vulgare	2110	0.97
O. dictamnus	2470	0.92
M. pulegium	4520	0.95
L. stoechas	5230	0.98
M. officinalis	9150	0.88

L-carvone EC50/1d= 120 µg/ml
pulegone EC50/1d= 160 µg/ml

$EC_{50/1d}$ (*M. incognita*)
2-undecanone=20.6 µg/ml



***Fosthiazate*: 0.4 µg/ml**

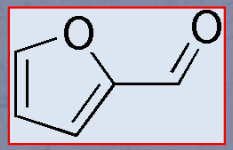
Ntalli et al., Pest Mang Sci 2011, 67: 341-351 ; Ntalli et al., 2010, 58: 7856-7863

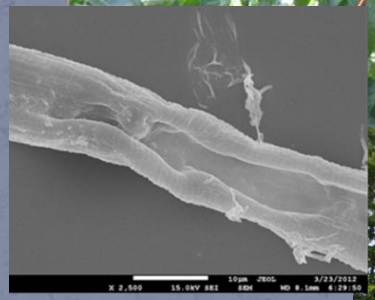
Melia azedarach, Ailanthus altissima – aldehydes

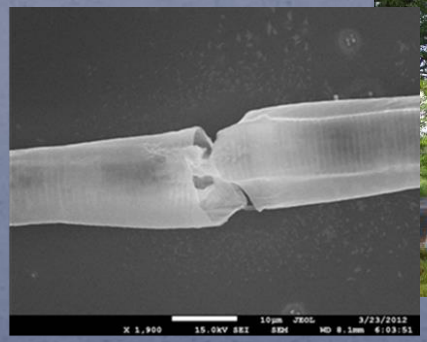
$EC_{50/1d}$ (*M. incognita*)

Furfural = 11 µg/ml

Fumigant activity 24 µg/mL

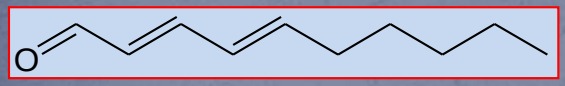




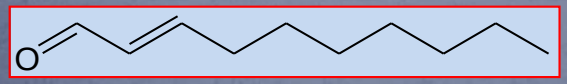


$EC_{50/1d}$ (*M. javanica*)

E,E)- 2,4-decadienal= 11.7 µg/mL



(E)-2-decenal = 21.79 µg/mL



Fosthiazate: 0.4 µg/mL

Ntalli et al., J Agr Food Chem 2010, 58: 11390-11394; Caboni et al., J Agr Food Chem 2012, 60: 1146-1151

Melia azedarach – acids

EC_{50} (*M. incognita*)

	µg/mL (1 h)	µg/mL (24 h)
Acetic acid	64	38.3
Butyric acid	69.8	40.7
Hexanoic acid	88.7	41.1
Decanoic acid	353.7	248.3
Fosthiazate	3.3	0.4

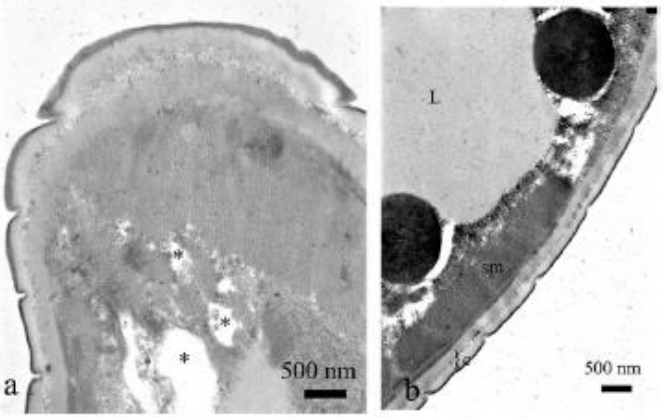


FIG. 4. Cross section of the nematode treated with acetic acid, 20 µg·mL^{-1}. Note A. electron-lucent regions close to the muscle layer (*), which were not present within B. control. sm = somatic muscles, c = cuticle, L = lipid granules.

Ntalli et al., J Agr Food Chem 2010, 58: 11390-11394; Ntalli et al., J Nem 2016, 48: 248-260

Parsley (*Petroselinum crispum*)

furanocoumarins

$EC_{50/1d}$ (*M. incognita*)

Xanthotoxol = 68 µg/mL

Psoralen = 147 µg/mL

Xanthotoxin = 200 µg/mL

Fosthiazate: 0.4 µg/mL

Caboni et al., Pest Man Sci, 2014, 60: 1146-1151

Rucola (*Eruca sativa*) – isothiocyanates

$EC_{50/1d}$ (*M. incognita*)

erucin = 3.2 µg/ml
penthyl isothiocyanate = 11.1 µg/mL
hexyl isothiocyanate = 11.3 µg/mL
(E)-2-hexenal = 15 µg/mL
2-ethyfuran = 16 µg/mL
methyl thiocyanate = 18 µg/mL

Fosthiazate: 0.4 µg/mL

Aissani et al., J Agr Food Chem, 2015, 63: 6120-6125

Isothiocyanates mode of action

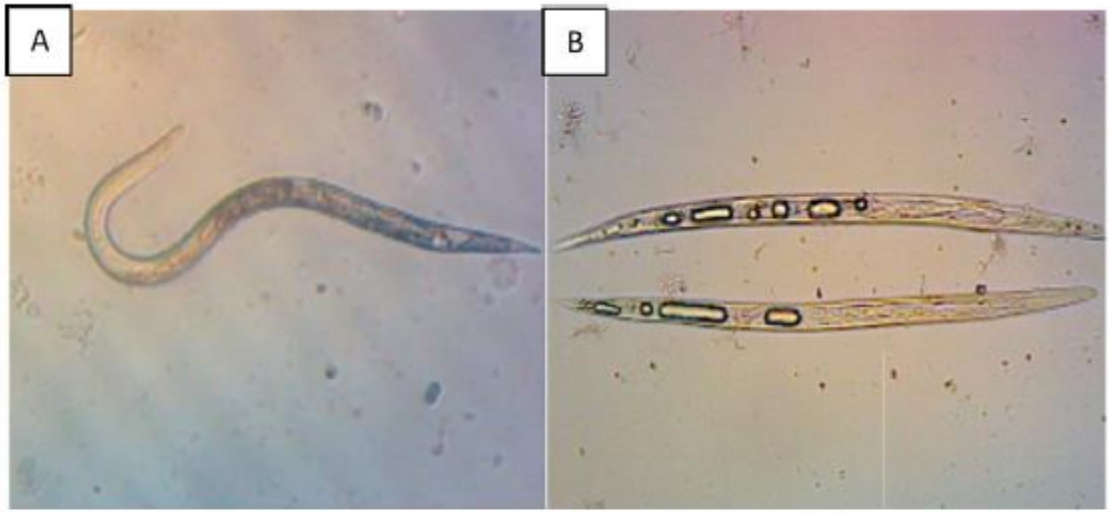


Figure 2. *M. incognita* J2 before (A) and after (B) a 24 h immersion in erucin at 10 mg/L. After treatment, nematodes were paralyzed in a straight shape, and internal vacuoles were evident.

Aissani et al., J Agr Food Chem, 2015, 63: 6120-6125

Botanical Nematicides
Perspectives of Authorization

Alternative PPPs – *botanicals*

Regulation in non-EU countries – Canada

Biopesticides: Information Required for Assessment of Value

DIR2012-01 (efficacy, effect on host organisms in connection with its intended use(s), health, safety and environmental benefits and social and economic impact)

Regulation EU

- **Basic Substances:** art.23 Regulation (EC) 1107/2009 (no claims as PPPs)
- **Low risk a.i. & PPPs:**

  art.22 Reg 1107/2009; PP 1/296 (1) (market authorisation as PPPs)

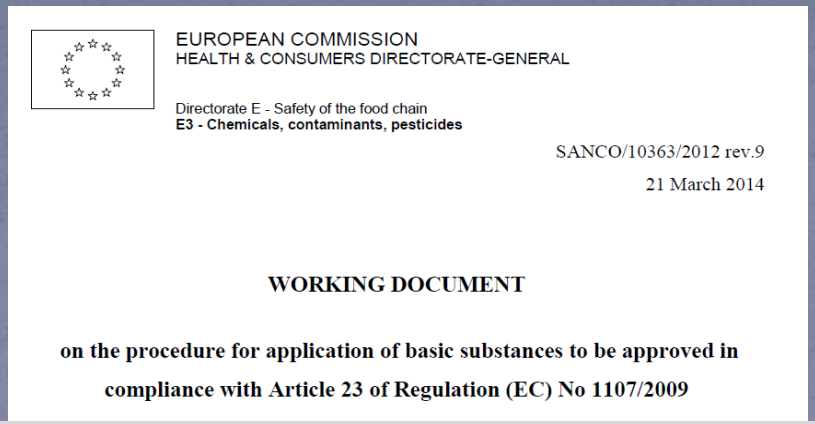
Basic substance – Definition

a.i. that are not used as PPPs, since the economic turnover of registration costs is low, but of use in plant protection (Καν. (EC) 1107/2009).

- α) not a substance of concern, according to art.3(4) Reg. (EC) 1107/2209
- β) of no inherent capacity to cause endocrine disrupting, neurotoxic or immunotoxic effects; and
- **γ) not predominantly used for plant protection purposes but nevertheless is useful in plant protection either directly or in a product consisting of the substance and a simple diluent; and**
- **δ) not placed on the market as a plant protection product.**

An active substance which fulfils the criteria of a 'foodstuff' as defined in Article 2 of Regulation (EC) No 178/2002 shall be considered as a basic substance.

Application: Basic substance



EUROPEAN COMMISSION
HEALTH & CONSUMERS DIRECTORATE-GENERAL

Directorate E - Safety of the food chain
E3 - Chemicals, contaminants, pesticides

SANCO/10363/2012 rev.9

21 March 2014

WORKING DOCUMENT

on the procedure for application of basic substances to be approved in compliance with Article 23 of Regulation (EC) No 1107/2009

- **The application is handed to the EU my the member state or the applicant**
- **Unlimited validity period**
- No authorization is required for the use of products consisting of only basic substances. However, these products are not marketed as PPPs.
- The member states and the EU must inform the public about the approvals of basic substances (listing according to Καν. (EC) 540/2011, site EU and member states

After the authorization of a basic substance, the farmer may apply it to the crop for plant protection purposes under his responsibility following the instructions in the authorization..

Basic substances allowed in organic agriculture comply with two criteria: i) they are of vegetable or animal origin ii) they are considered to be "foodstuff" (Annex II of Commission Regulation (EC) No 889/2008).

Active substance
Azadirachtin extracted from *Azadirachta indica* (Neem tree)
Beeswax
Gelatine
Hydrolysed proteins
Lecithin
Plant oils (e.g. mint oil, clove oil, citronella oil)
Pyrethrins extracted from *Chrysanthemum cinerariaefolium*
Micro-organisms (bacteria, viruses and fungi)
Spinosad
Diammonium phosphate
Pheromones
Pyrethroids (only deltamethrin or lambdacyhalothrin)
Ferric phosphate (iron (III) orthophosphate)

Copper in the form of copper hydroxide, copper oxychloride, (tribasic) copper sulphate, cuprous oxide, copper octanoate
Ethylene
Fatty acid potassium salt (soft soap)
Potassium aluminium (aluminium sulphate) (Kalinite)
Lime sulphur (calcium polysulphide)
Paraffin oil
Mineral oils
Quartz sand
Sulphur
Calcium hydroxide
Potassium bicarbonate
Basic substances

Mustard seed powder – fungicide

APPENDIX I

Identity and biological properties

MUSTARD SEEDS POWDER

Common name (ISO)	There is no ISO common name for this substance
Chemical name (IUPAC)	Not relevant, the substance is a complex mixture
Chemical name (CA)	Not relevant, the substance is a complex mixture
Botanical classification	*Sinapis alba (Brassica alba), Brassica juncea* and *Brassica nigra.*
Common names	White mustard, Indian mustard, Chinese mustard, black mustard seed powder
CAS No	84929-33-9 (Brassica alba seed extract) 93062-78-3 (Brassica juncea seed extract) 90064-15-6 (Brassica nigra seed extract)
CIPAC No and EEC No	284-517-9 (Brassica alba seed extract) 296-833-4 (Brassica juncea seed extract) 290-076-3 (Brassica nigra seed extract)
FAO specification	Not available
Minimum purity	Not relevant
Relevant impurities	Purity is depending on the origin
Molecular mass and structural formula	none
Mode of Use	Mix 1,5 kg of mustard seeds powder with 4,5 L water. Treat 100 kg seeds with the slurry created.
Preparation to be used	Water dispersible powder for slurry seed treatment (WS)
Function of plant protection	Fungicide for seed treatment

Low Risk a.i. & PPPs

- **Guidance documents:**

- Dir. (EC) 1107/2009, art 22
- Dir. (EU) 2017/1432 (criteria for the approval of low-risk active substances)
- EPPO PP1/296(1) (Principles of efficacy evaluation for low-risk PPPs)

- **Reduced requirements in:**

- Efficacy trials (number of GEP trials, 1 -2 years of trials in EPPO zones)
- Accepted medium efficacy and use of an untreated control is enough. - ***Compensatory benefits***: application in different host growth stages, not toxic to non-target organisms, compatibility with IPM
- Resistance management: complex mixtures of compounds with different mode of action

Conclusions

- **Soil incorporation of botanical material prior to the establishment of the sensitive culture:**
  - parsley, rucola(crop rotation for incorporation)
  - row orange peel (recycling of juicing solid wastes)
  - dry rosmary and levanta (recycling of aromatherapy solid wastes)
  - row pruning wastes chinaberry and ailanthus
- **Basic substances:** aqueous mint extract (edible)
- **Low risk active substances:**
  - **aqueous extracts:** mint, melia (?)
  - **Essential oils:** rucola
- **Plant Secondary Metabolites:** aldehydes, ketones, acids, isothyocyanates, fouranocumarines
  - g.g *trans*-anethole & geraniol,
  - (*E*,*E*)-2,4-decadienal & (*E*)-2-decenal

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Thank you for your attention.