Trifolio-M

An Applicant's Experience

NeemAzal®-T/S

A brief history of re-registration

- **Our aim is:**

the development of products for pest control on the basis of natural resources (natural a.i.'s from plants), which are compatible to organic farming...

and

the production and marketing of these products.

They should be:

biological **efficient** **ecological** **non-toxic**

The history...

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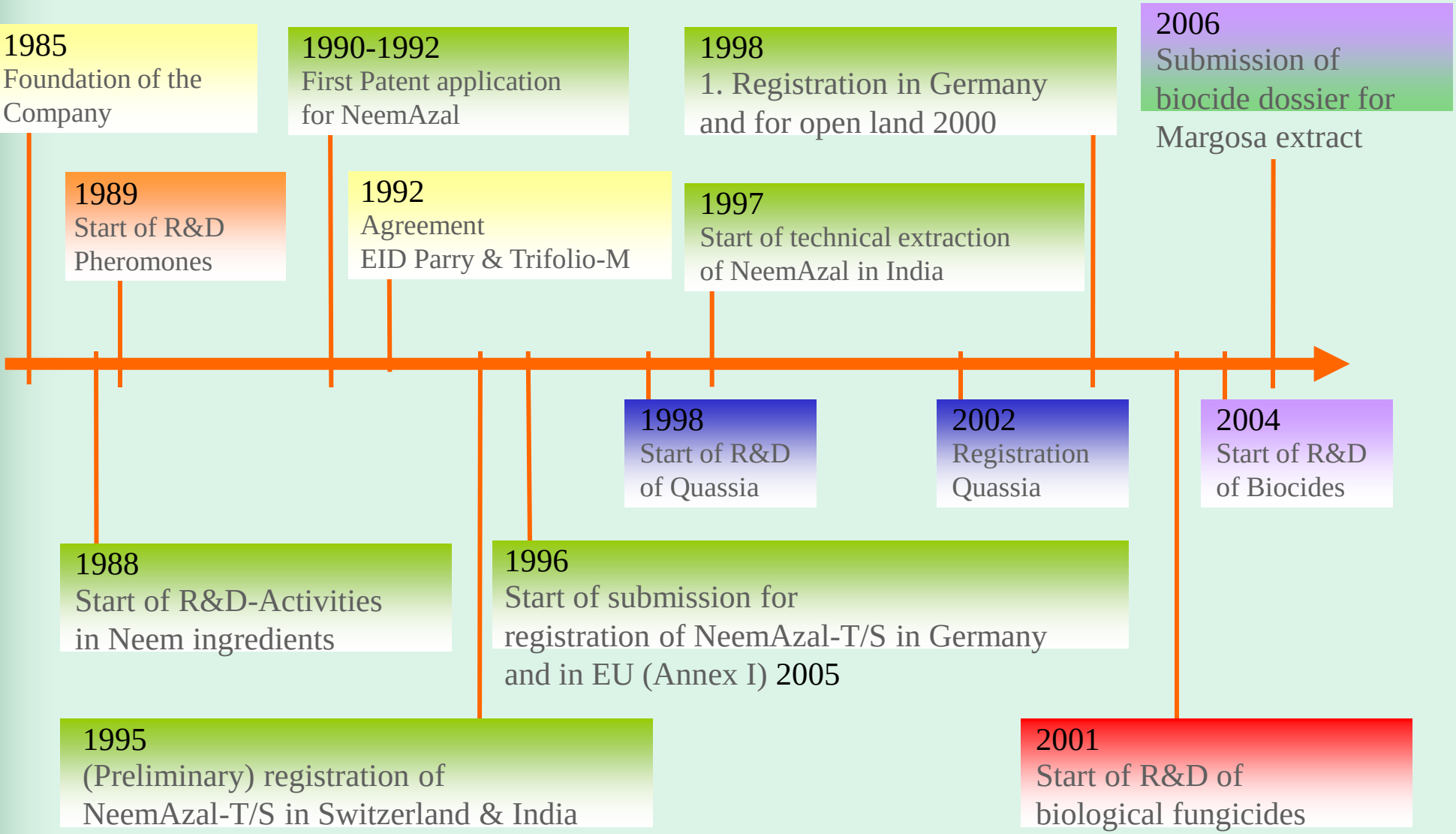

1992 - 2004 14 Workshops with over 1.500 participants from more than 80 countries

Requirements for plant protection products

1. Efficiency
2. Security in the success of application
3. Considerate influence on beneficial insects
4. Minimisation of the appearance of resistancies
5. Toxicological safety for the user
6. Toxicological safety for the consumer
7. Environmental safety (biodegradability, no accumulation)
8. Toxicologically and environmentally safe production
9. Inexpensive production*
10. World-wide availability of standard quality

* including social cost (drinking water analysis and purification, environmental pollution etc.)

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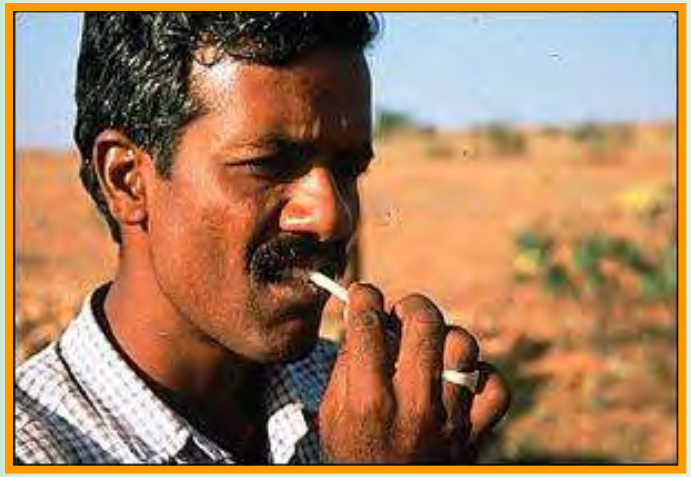
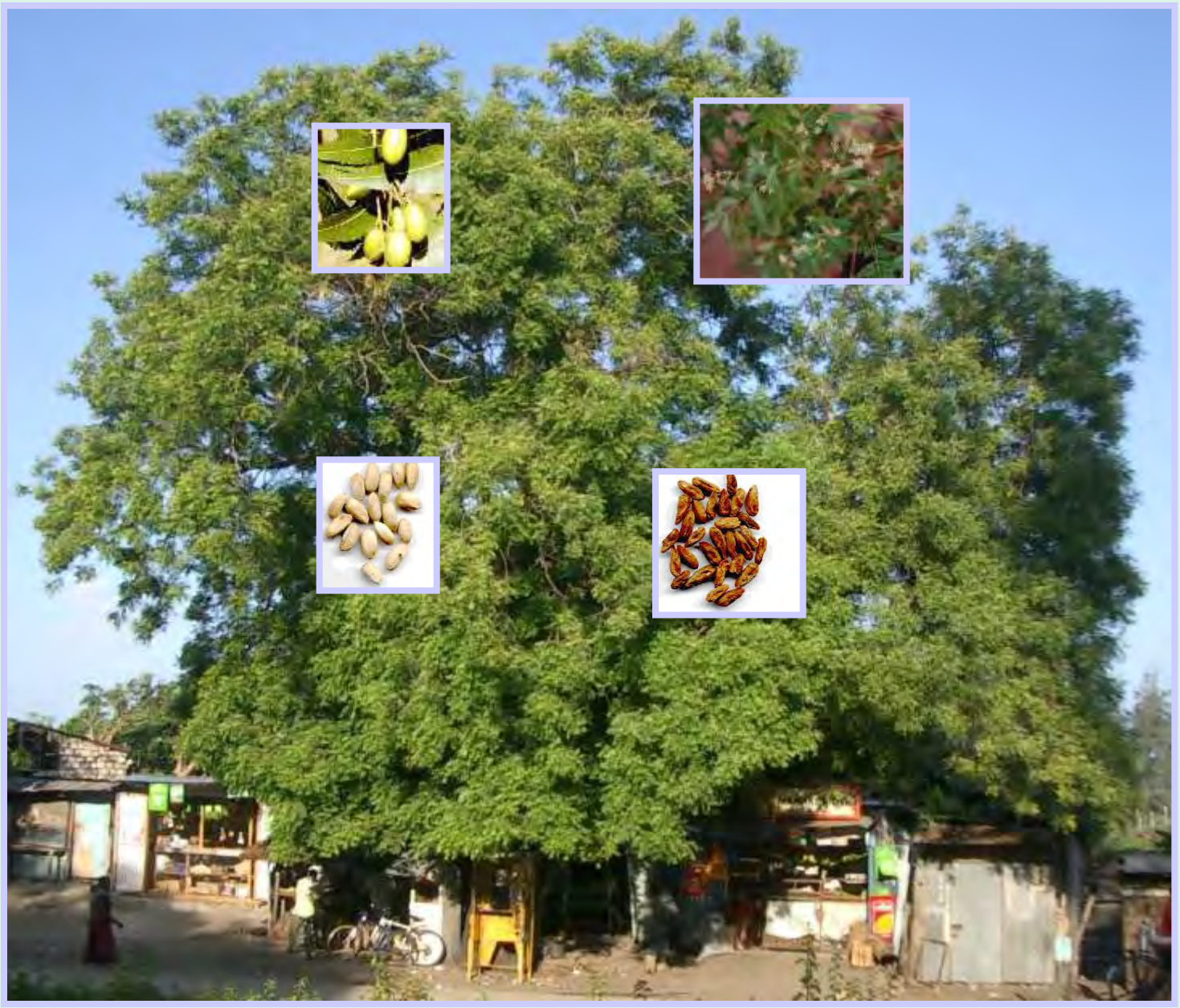
Azadirachta indica – Indian Neemtree



Azadirachta indica – Indian Neemtree



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Neem Extraction Process yields NeemAzal technical

Neem fruit
(100 kg)

Neem seeds
(20 kg)

Seed kernels
(10 kg)

Extraction Process

NeemAzal®
(0,1 kg)
34% Azadirachtin A

Neem oil
(4 kg)

Neem cake
(5 kg)

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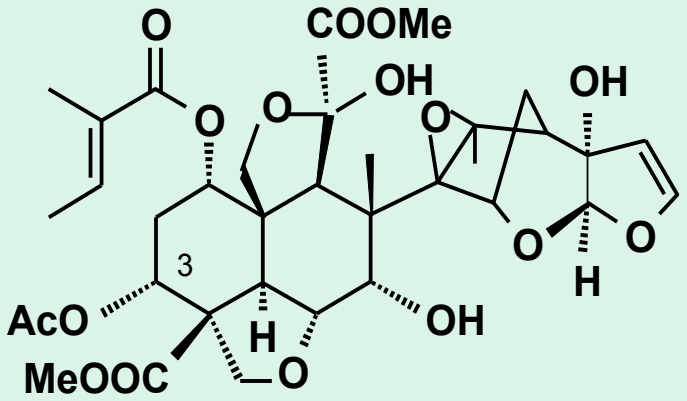
Composition of the formulation

NeemAzal-T/S (EC):

- **Active ingredient (a.i.): Azadirachtin**
  - **Standardised natural Neem extract (approx. 3% NeemAzal technical)**
  - **Containing Azadirachtins and other limonoids**
- **Analytical lead compound:**
  - **Azadirachtin A (AzA) (in NeemAzal-T/S standardised to 1%)**
    - ***1H,7H-Naphtho[1,8-bc:4,4a-c´]difuran-5,10a(8H)-dicarbocylic acid,10-(acetyloxy)octahydro-3,5-dihydroxy-4-methyl-8-[(2-methyl-1-oxo-2-butenyl)oxy]-4-(3a,6a,7,7a-tetrahydro-6a-hydroxy-7a-methyl-2,7-methanofuro[2,3-b]oxireno[e]oxepin-1a(2H)-yl)-dimethylester,[2aR-[2aa, 3b, 4b(1aR*, 2S*, 3aS*, 6aS*, 7S*, 7aS*), 4ab, 5a, 7aS*, 8b(E), 10b, 10aa, 10bb)]***



Composition of NeemAzal technical

Substance	**av. content in NeemAzal By weight (%)**
Azadirachtins:	
Azadirachtin A	34
Azadirachtin B	approx. 5.5
Azadirachtin D	aprrox. 2.1
Azadirachtin E	≤ 1
Azadirachtin F	≤ 1
Azadirachtin G	≤ 1
Azadirachtin H	approx. 2.3
Azadirachtin I	approx. 0.8
Azadirachtin K and other Azadirachtins	≤ 2
Azadirachtinin	approx. 2
Sum of Azadirachtins:	**51,7**

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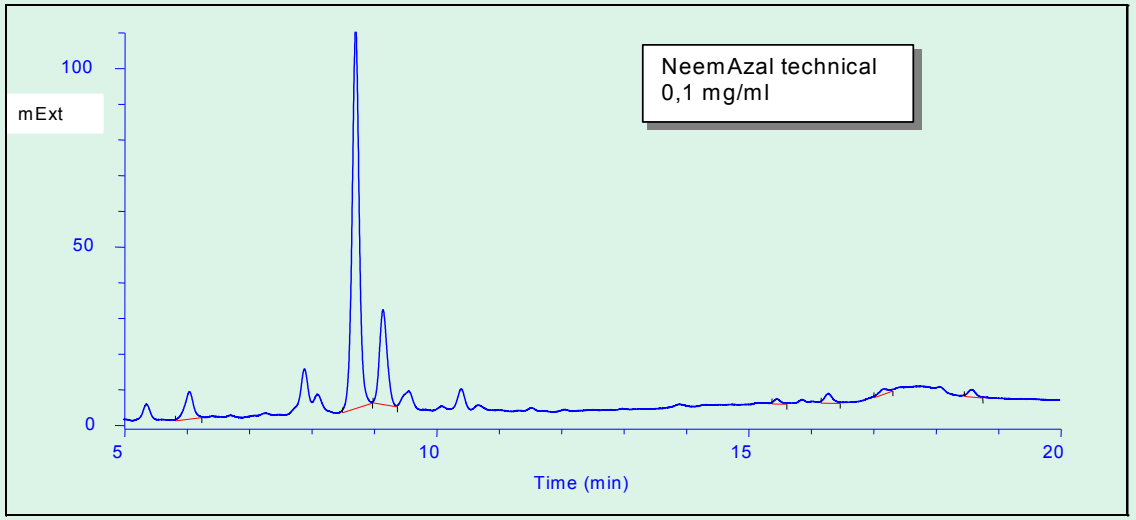
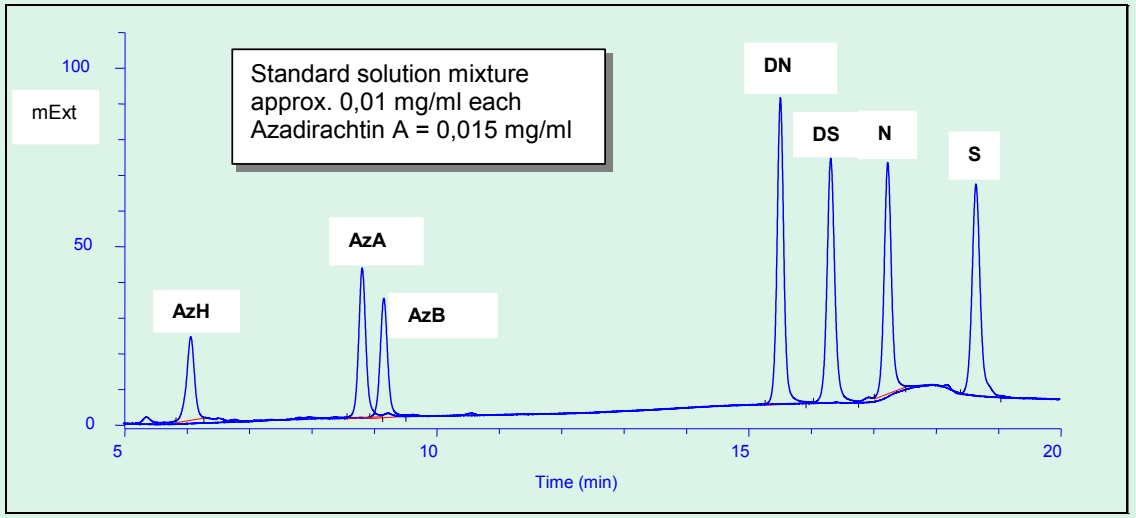
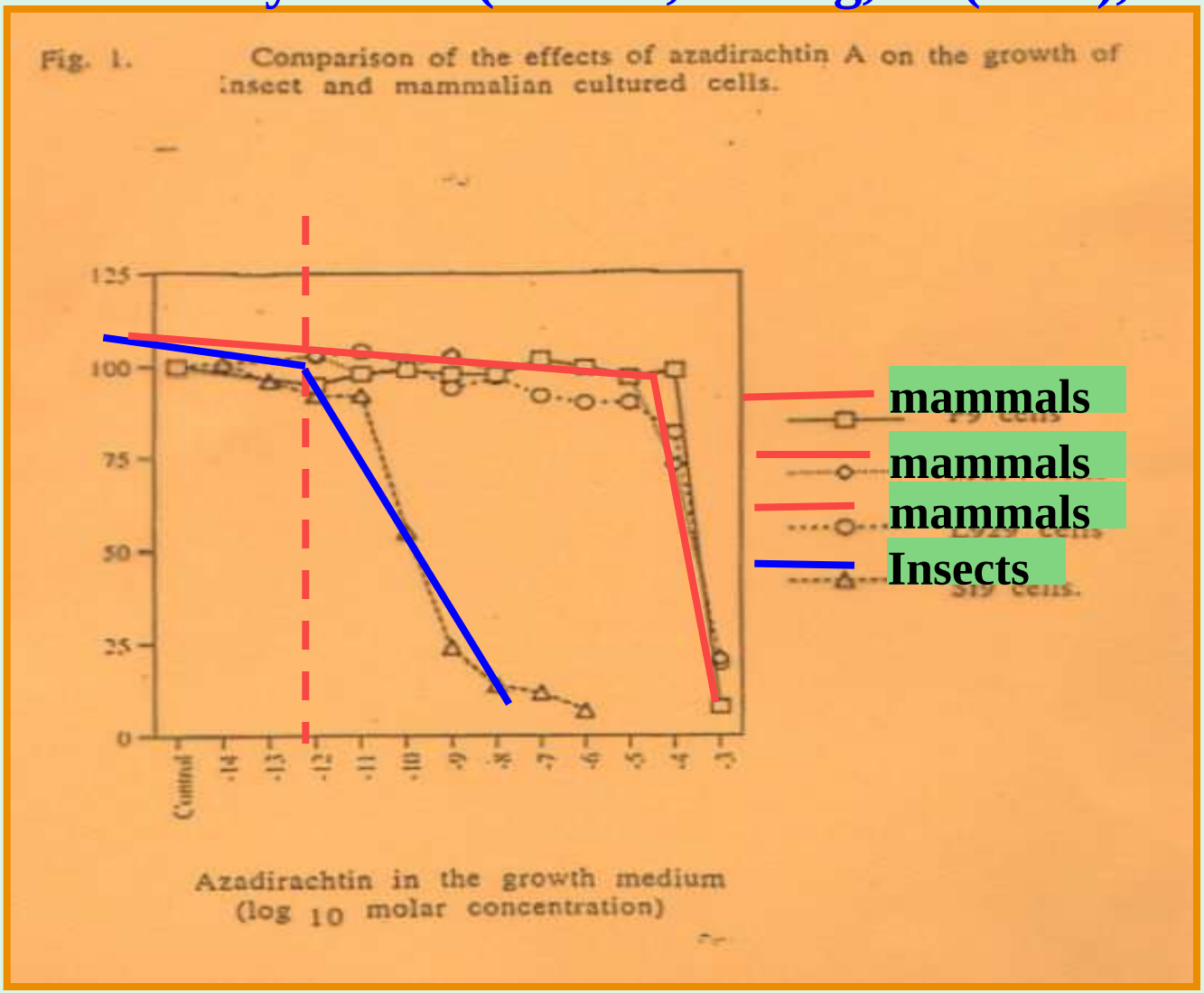
Analytics





Azadirachta indica – Indischer Neembaum

Insectcells react to Azadirachtin at a concentration of 10^{-11}, but mamals only at 10^{-4} (Jabbar, Strang, R. (1997), WS VII)



Mode of action & target pests: NeemAzal-T/S (ec)

Effects on the hormonal system of insects

1. feeding inhibition
2. moulting inhibition
3. fertility reduction
4. mortality

Azadirachta indica

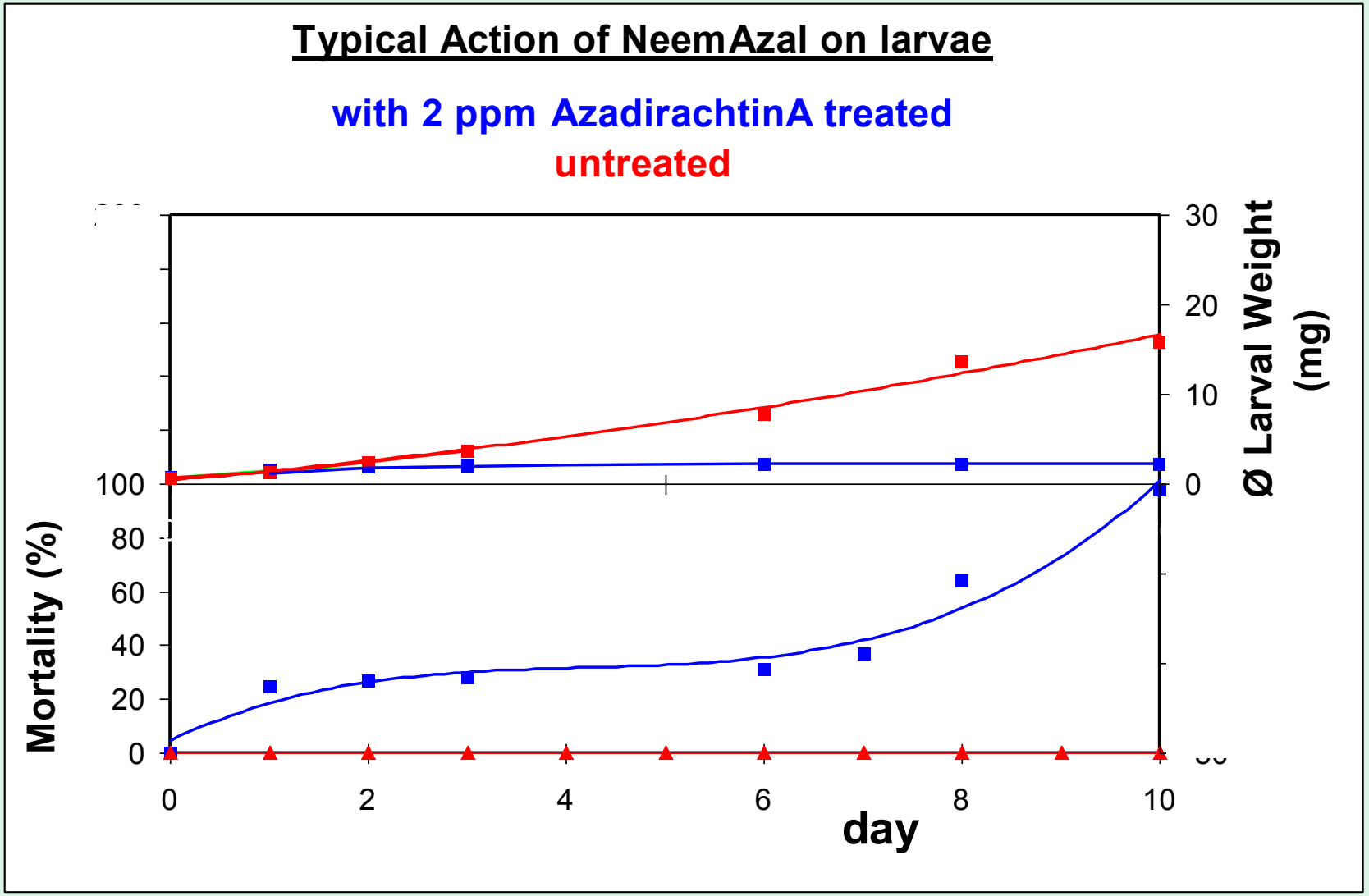
NeemAzal-T/S

Mode of action: oral uptake

by larvae:

- feeding inhibition

Feeding inhibition



Mode of action:

2. moulting inhibition (after a few days)

A Normal Larva

Normal larva

after treatment with NeemAzal-T/S

Mode of action:

3. Fertility reduction (next generation)

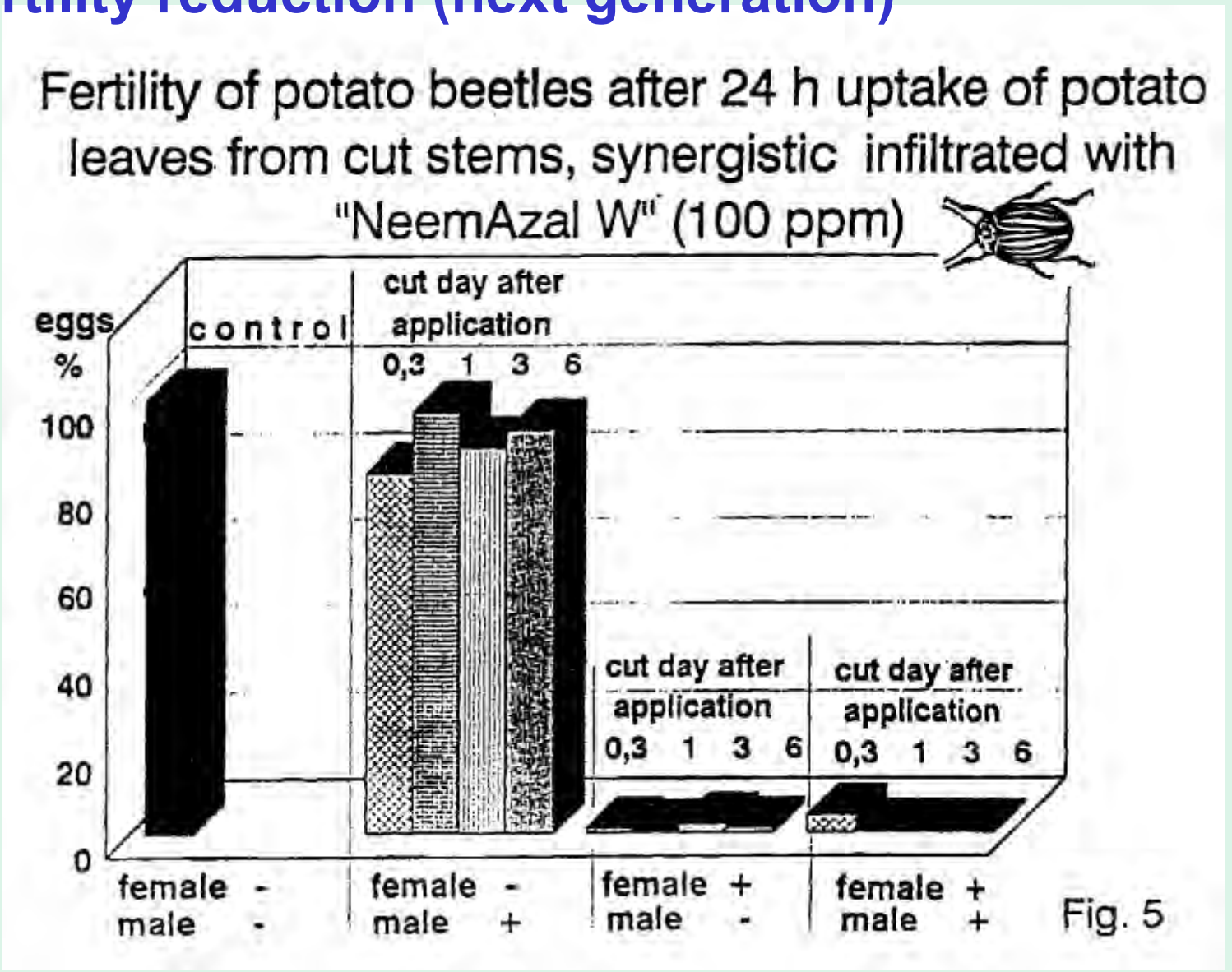
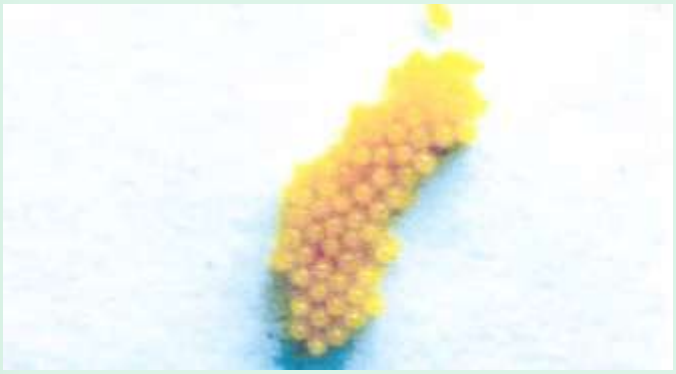


Fig. 5

Mode of action:

3. Fertility reduction (next generation)



Egg deposit (untreated)

„Egg deposit“ after treatment

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Slow mechanism of action ⇒ 3. Fecundity inhibition – adult (Wagenhoff)

Cockchafer Melolontha hippocastani

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Use of new NeemAzal-Formulations against *Cameraria ohridella* (Lep., Gracillariidae) (Pavela, 2005)

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Anwendung im Obstbau an Beerenobst - Morgan, Sweden 2004

Gelbe Stachelbeerblattwespe *Nematus ribesii* (Hym., Tenthredinidae)

Behandlung: - 1-

2x gegen jungen Larven

- 400 ml NeemAzal-T/S /100 l Wasser/ha

ecological efficient biological

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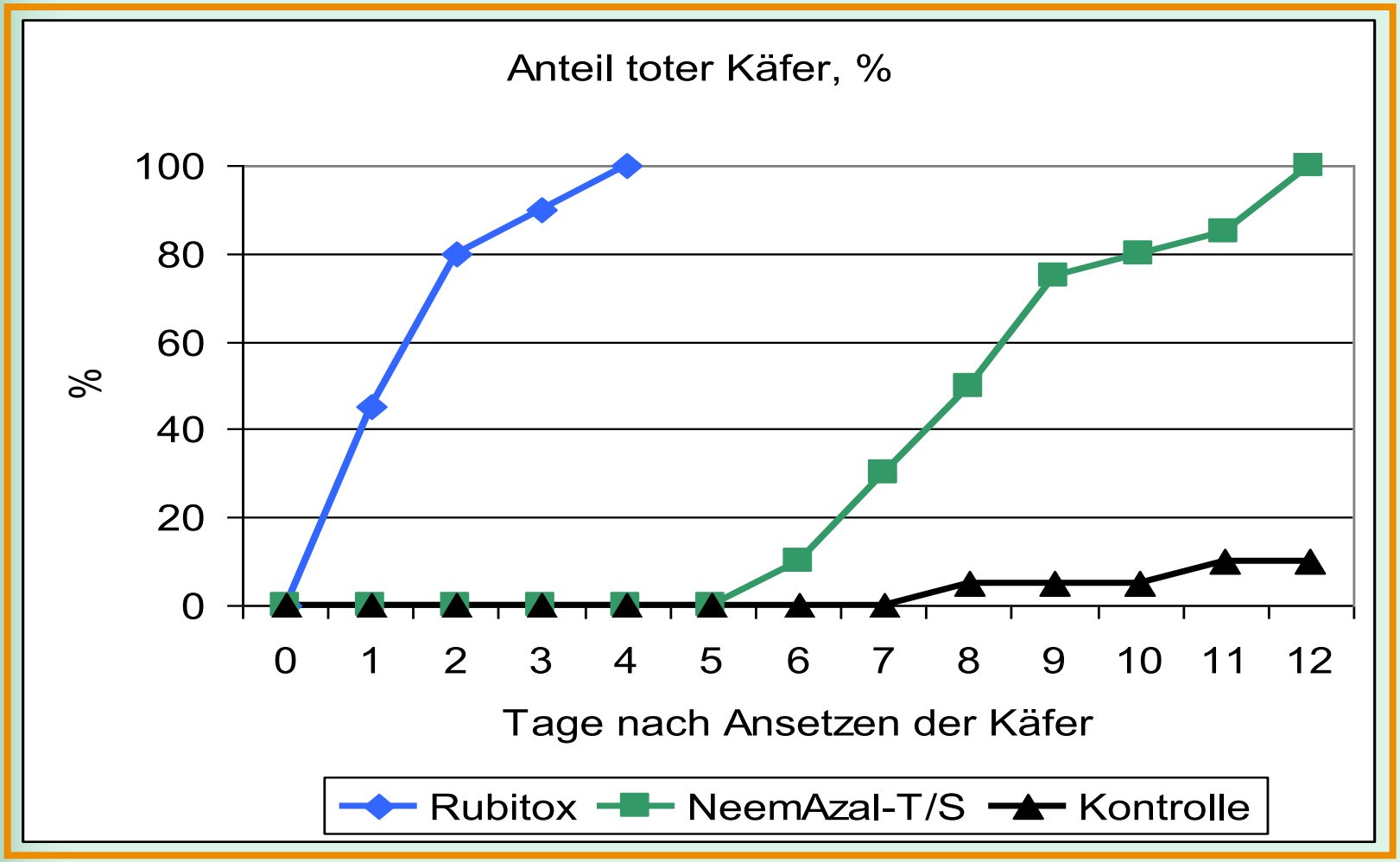
Mortality of adult beetles in the Lab (Schröter, FVFA, Freiburg)

Cochafer *Melolonta hippocastani*



Mode of action & target pests: NeemAzal-T/S (ec)

Especially control of sucking and biting insects as well as mites

- aphids
- caterpillars
- white flies
- thrips
- miners
- beetles, and other insects and
- mites

Resistance management

Resistance – Feng, Isman, 1995

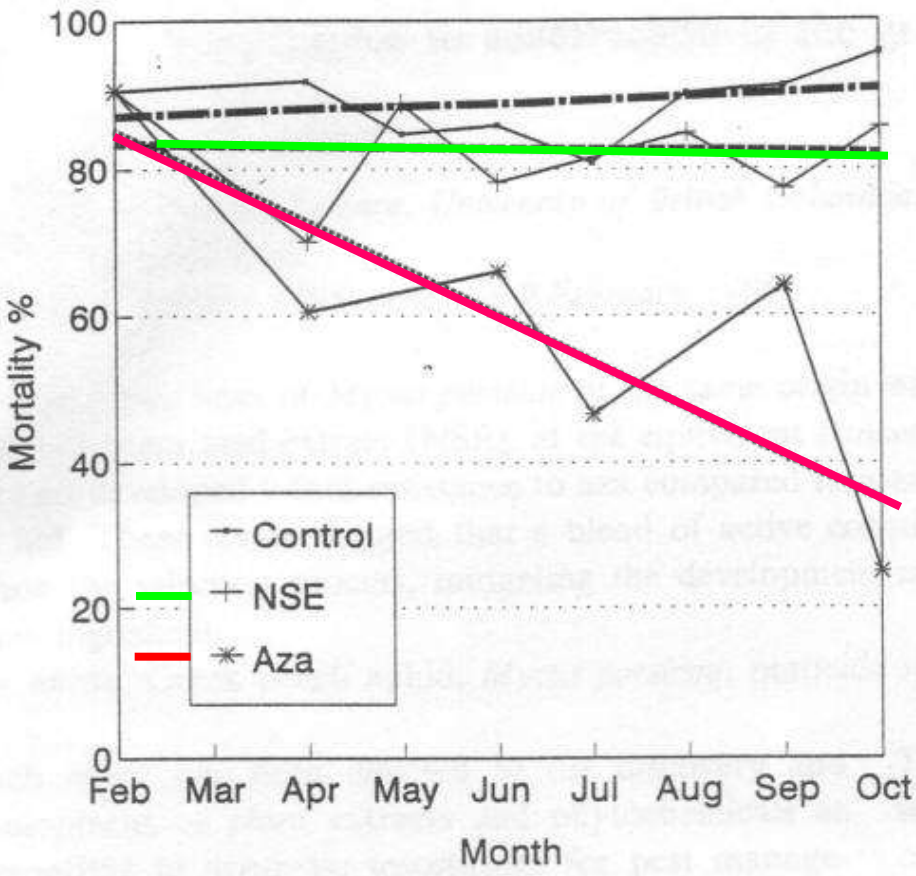


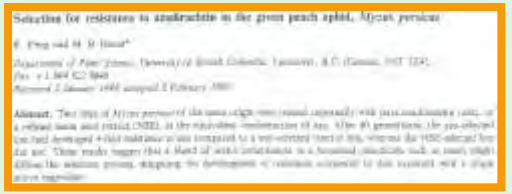
Figure. Susceptibility to a discriminating concentration of azadirachtin (8 ppm) in control, NSE-selected and aza-selected *M. persicae* lines in leaf disc assays. Dashed lines represent regression lines for mortality data versus date of bioassay.

Results:

- 40 generations *Myzus persicae (Hom., Aphididae)*

-9x resistence of AzA pur

- NO after using of NSE



Resistance – Abdullah, 1999

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Results:

- 12 Generations of *Spodoptera exigua* (Lep., Noctuidae) and use LD50-Dose

-Cypermethrin 95,8x

-*Bacillus thuringiensis* 5,6x resistance rate

- 1,1-1,44x after using of NSKE

Table 1 Selection and progression of resistance to cypermethrin of 3rd instar larvae of beet armyworm under laboratory condition for 12 generations in 1999.

Generations	Non-selection strain LC_{50} (ppm)	Selection strain LC_{50} (ppm)	Resistance ratio (RR)[1]
F_1	251	383	1.53
F_2	200	805	4.03
F_3	176	1237	7.03
F_4	172	1677	9.75
			17.45
			20.57
			32.23
			46.76
			52.21
			74.50
			83.68
			95.83

Table 3 Selection and progression of resistance to *Bacillus thuringiensis* var. *kurstaki* of 3rd instar larvae of beet armyworm under laboratory condition for 12 generations in 1999.

Generations	Non-selection strain LC_{50} ($\times 10^6$SU/L)	Selection strain LC_{50} ($\times 10^6$SU/L)	Resistance ratio (RR)[1]
F_1	7.3	7.6	1.04
F_2	11.2	12.3	1.10
			1.30
			1.18
			1.33
			1.77
			1.82
			2.01
			2.61
			2.71
			3.97
			5.60

Table 2 Selection and progression of resistance to neem extract of 3rd instar larvae of beet armyworm under laboratory condition for 12 generations in 1999.

Generations	Non-selection strain LC_{50} (ppm)	Selection strain LC_{50} (ppm)	Resistance ratio (RR)[1]
F_1	3.98	4.38	1.10
F_2	6.13	6.32	1.03
F_3	6.56	7.14	1.09
F_4	8.35	8.20	0.98
F_5	8.97	10.36	1.15
F_6	8.46	9.81	1.16
F_7	8.76	9.53	1.09
F_8	9.14	11.09	1.21
F_9	9.08	11.78	1.30
F_{10}	8.36	11.74	1.40
F_{11}	9.99	13.04	1.31
F_{12}	8.23	11.87	1.44

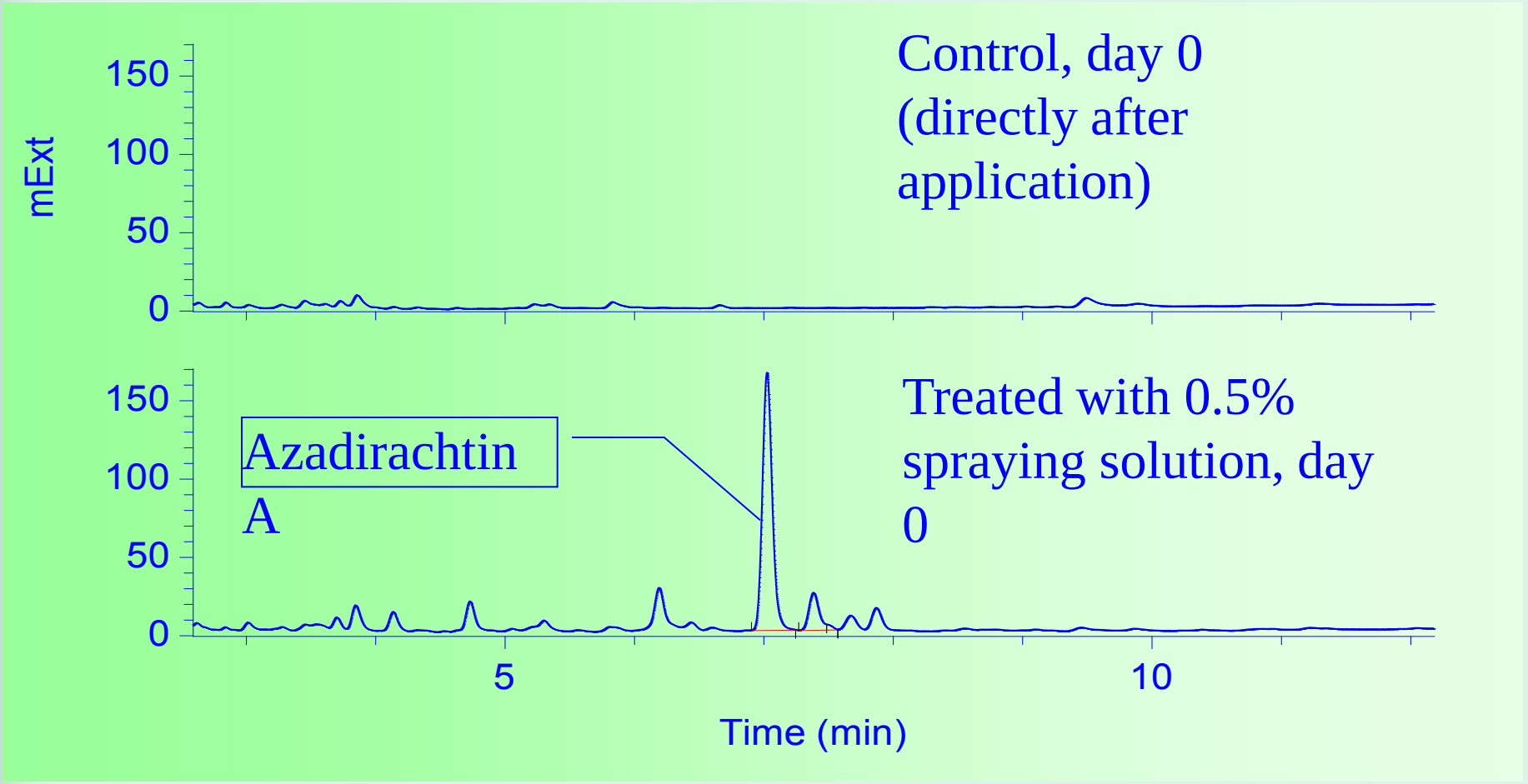
[1] LC_{50} of selection strain / LC_{50} of non-selection strain.

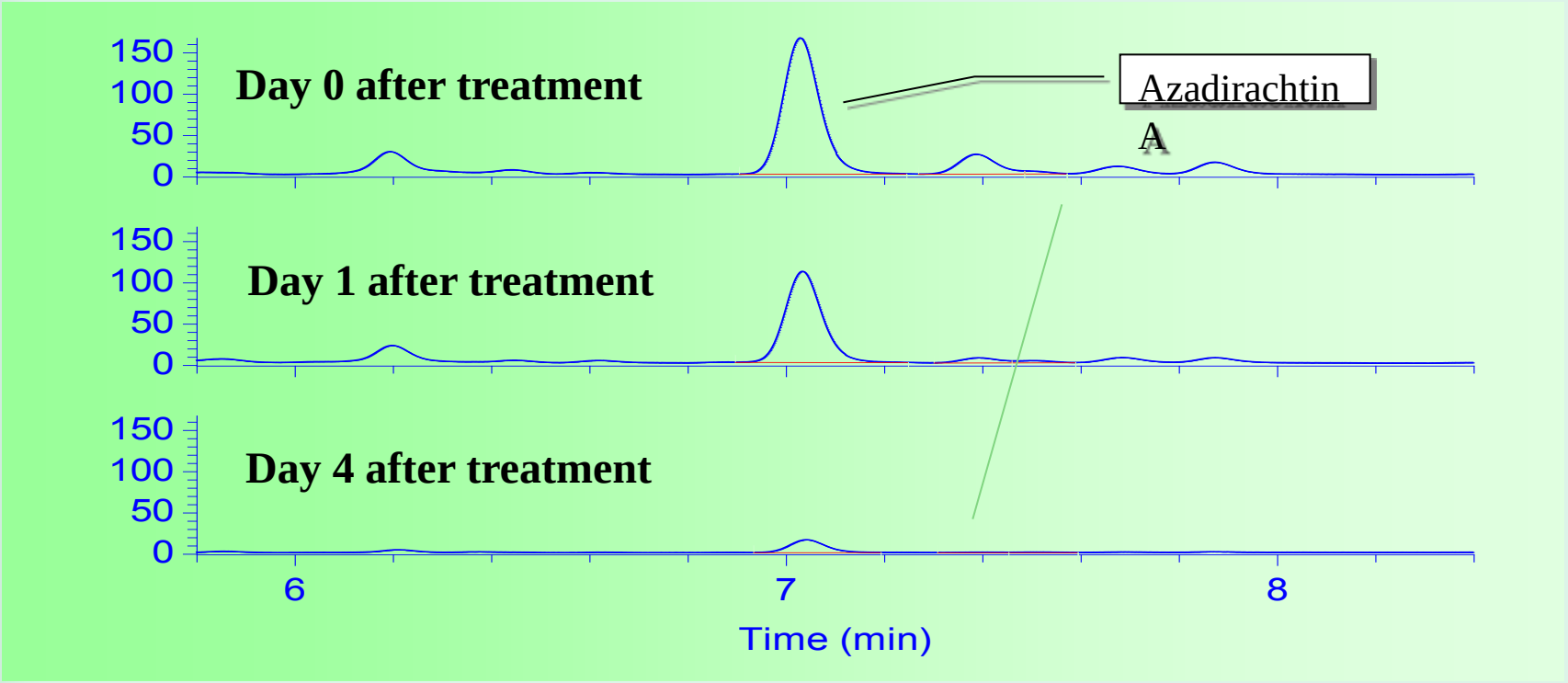
Monitoring Insecticide Resistance Development in Beet Armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

Md. Abdullah[1], Onob Saowthee[1], Siripan Tanlakorn[1], Somchai Sichaibut[2] and Sudawan Chareonviriyaphap[2]

Residue situation

AzA residues in spinach





HPLC chromatogram details of spinach, treated with NeemAzal-T/S

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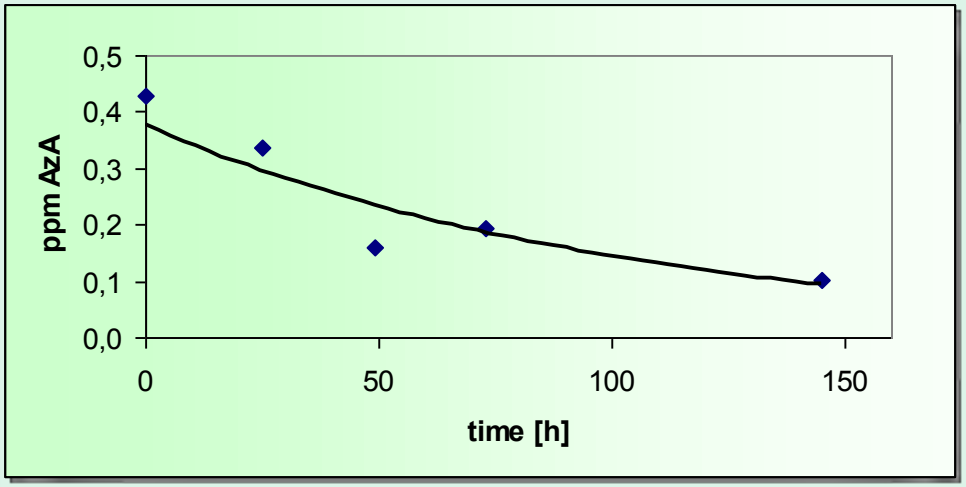
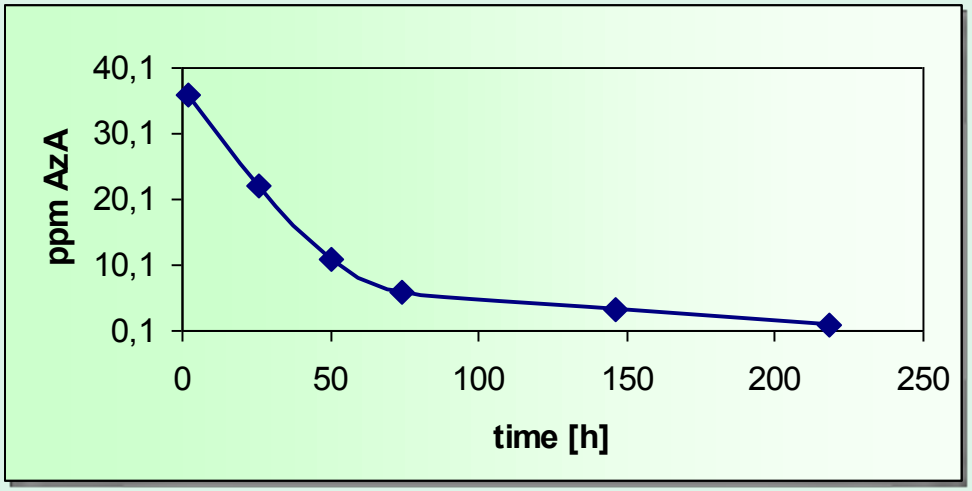
Degradation of Azadirachtin A (AzA) after 10fold application of NATS



AzA on/in tomatoes

$t_{1/2}$ = 3 days



AzA on/in tomato leaves

$t_{1/2}$ = 1 day

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Residues

Residue Studies (confidential information) BR / 04.06.2009

Crop	Crop growth stage at application	LOQ [mg AzA / kg]	AzA content[1] [mg AzA / kg]	MRL[2] [mg AzA / kg]	waiting period[3] [d]
Fruits:					
Apple	BBCH 87-89	0.1	< LOQ	1	0
Apple	BBCH 56-69	0.02	< LOQ		
Cherry	BBCH 81-87	0.02	0.26	1	0
Orange, peel and pulp	BBCH 87-98	0.02	peel: 0.055	0.5	0
			pulp: < LOQ	0.5	0
Peach	BBCH 83-86	0.02	0.049	1	0
Strawberry	BBCH 84-87	0.02	0.032	1	0

1) mean AzA content

2) MRL according to regulation No 149/2008

3) proposal by Trifolio-M GmbH

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Rückstände

Residue Studies (confidential information) BR / 04.06.2009

Crop	Crop growth stage at application	LOQ [mg AzA / kg]	AzA content[1] [mg AzA / kg]	MRL[2] [mg AzA / kg]	waiting period[3] [d]
Vegetables:					
Cabbage	BBCH 48-49	0.02	0.020	1	0
Cucumber, field	BBCH 75-76	0.02	< LOQ	1	0
Cucumber, greenhouse	BBCH 69-89	0.02	< 0.020		
Head lettuce	height: 25 cm	0.02	0.13	1	0
Potato	BBCH 40-70	0.01	< LOQ	1	0
Spinach	BBCH 11-49	0.1	1.01	1	1
		0.02	0.86		
Sweet pepper	BBCH 72-76	0.02	0.17	1	0
Tomato, field	ripe fruit; BBCH 82-84	0.1	< 0.043* from study with 10fold application rate	1	0
Tomato, greenhouse	fruit producing		< LOQ		

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Rückstände

Residue Studies (confidential information) BR / 04.06.2009

Crop	Crop growth stage at application	LOQ [mg AzA / kg]	AzA content[1)] [mg AzA / kg]	MRL[2)] [mg AzA / kg]	waiting period[3)] [d]
Herbs:					
Basil	height: 40-45 cm	0.02	0.43	1	0
Dill, fresh	BBCH 45-49	0.02	0.7	1	1
Dill, dried		0.02	1.38		
Fennel seeds	BBCH 92	0.02	< LOQ	0.01	0
Lemon balm, dried	BBCH 49	0.02	4.60	1	3
Lemon balm, fresh		0.02	0.81		
Parsley, dried, field	height: 25 cm	0.02	6.84	1	3
Parsley, fresh, greenhouse	46-49; height: 15 cm	0.02	3.06	1	?[4)]
Parsley, fresh, field	height: 25 cm	0.02	1.39	1	1
Sage	height: 35 cm	0.02	1.04	1	7
Savory, fresh	not stated	0.02	1.43	1	1
Savory, dried		0.02	5.39		

4) results on degradation on/in parsley differ variably, see: overview - fresh parsley

Developmental cost of Biological Plant Protection Products (estimated) in Comparison to Synthetics (IVA Data 2006)

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Years	1	2	3	4	5	6	7	8	9	10	million Euro
Active ingredient **Chemistry** Formulation	Extraction/Fermentation - Synthesis	Lab-scale	Process development						Production*		2
			Development		Development of Packing				Production*		
Research **Biology** Development	Screening Lab/Greenhouse	Small plot trials	Field trials						Registration		2
DEGRADATION AND RESIDUES	plant, animal, soil, water, air								Registration		8
TOXICOLOGY	acute u. chronic toxicity, cancerogenicity, mutagenicity, teratogenicity, reproduction										
ECO-TOXICOLOGY	algae, daphnia, fish, birds, micro-organisms, bees, beneficials								Registration		
MILLION EURO	**2 (102)**			**10 (98)**							**12 (200)**
NUMBER OF SUBSTANCES	**5 – 10 (140000)**			**1 (1)**							

* without cost for production plants

Risk Estimation

„For NeemAzal technical or its commercial formulation NeemAzal-T/S, no evidence of neither acute toxicity nor reproductive effects was obtained in valid studies.

However, in no way can it be concluded that other neem products are generally devoid of a significant risk since NeemAzal is considered as safe.“

Niemann,L. (2001): Regulatory Data Requirements for Health Evaluation of Biological Plant Protection Products in: ‚Practice Oriented Results on Use of Plant Extracts and Pheromones in Integrated and Biological Pest Control‘, Proceedings of the 10th Workshop, p 95

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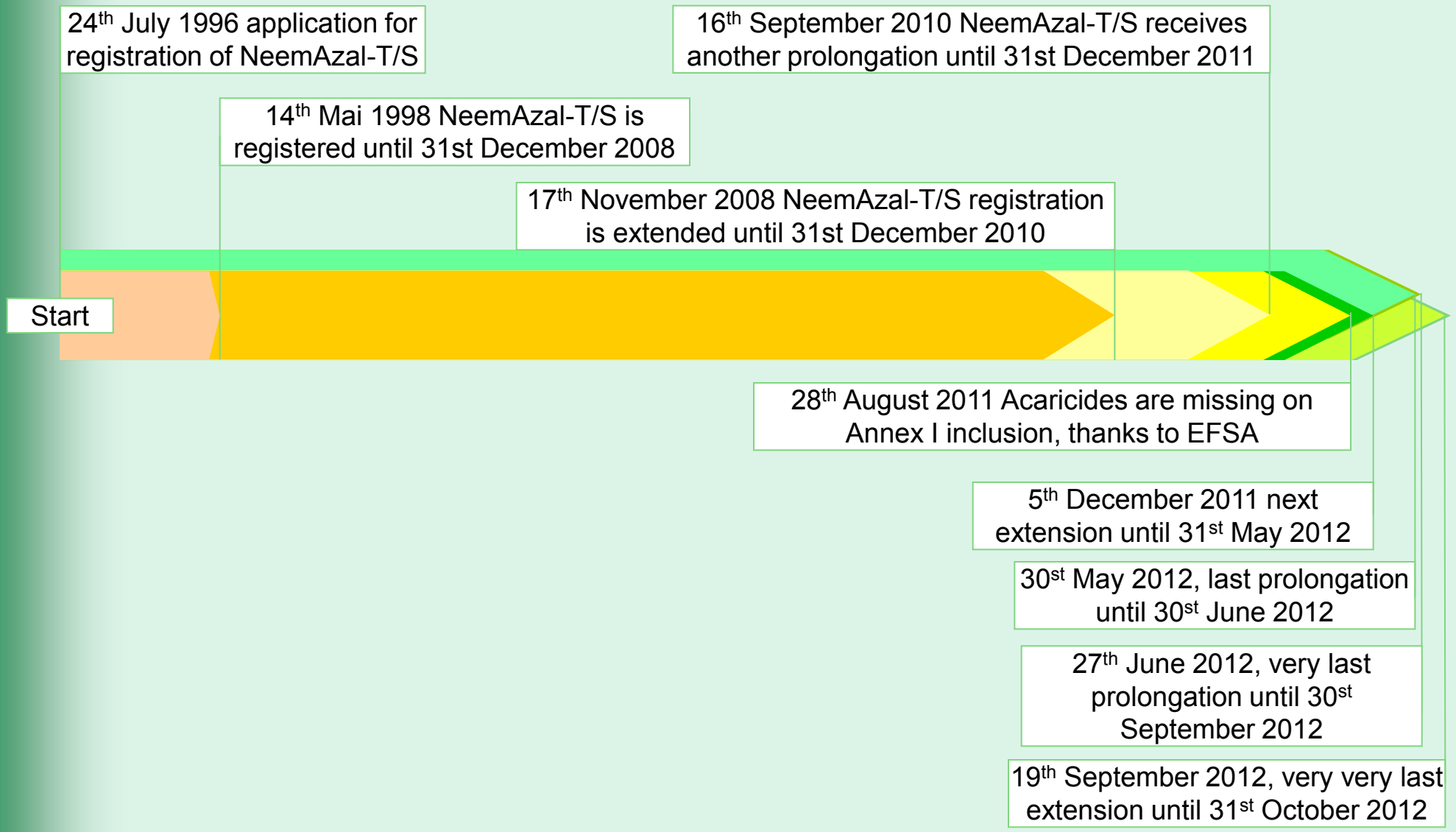
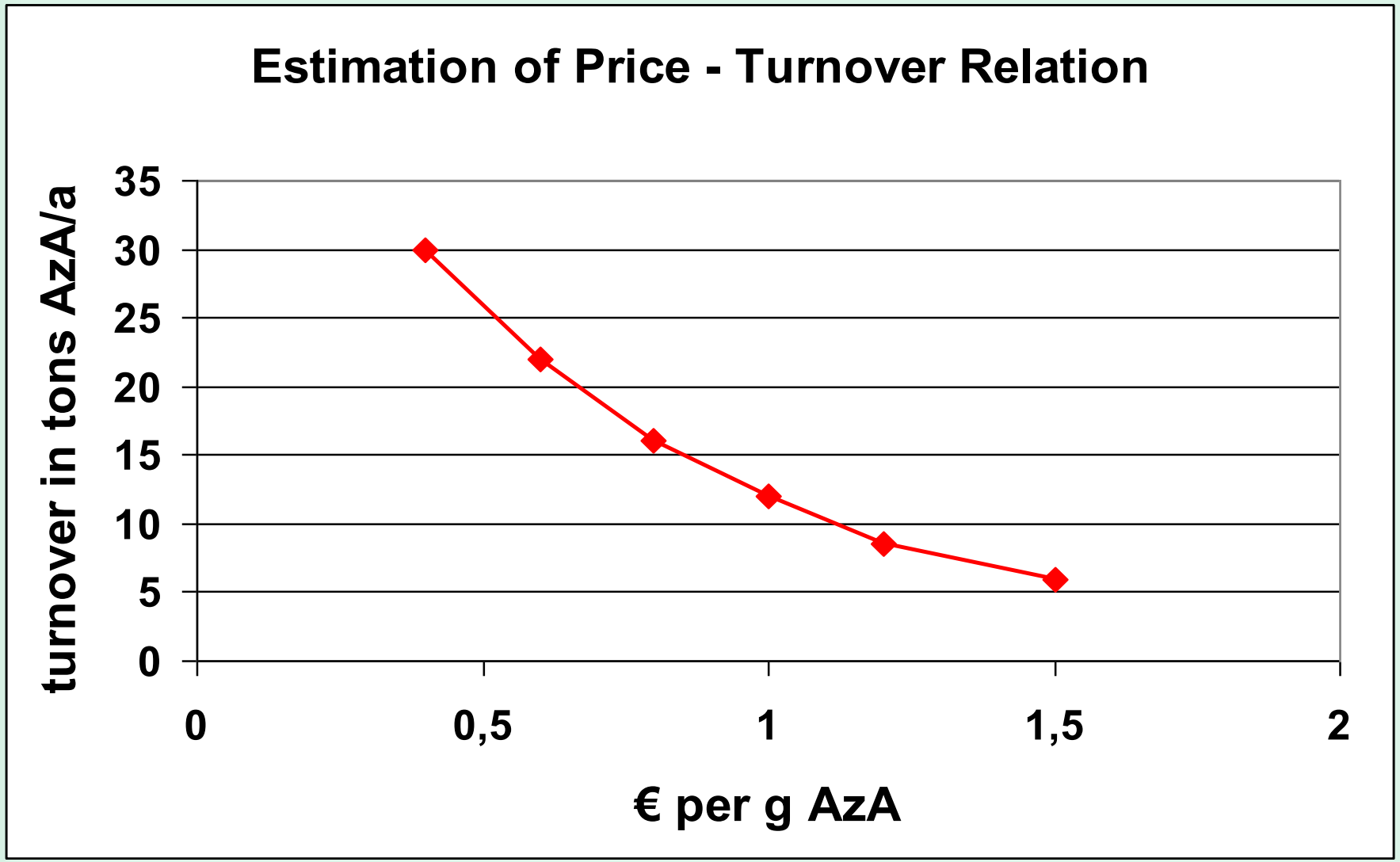
Timeline of registration in Germany



Registration dossier

1995

2007

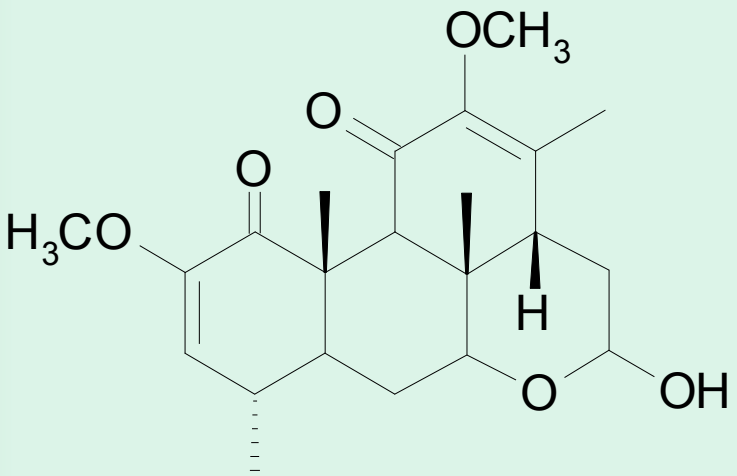

Estimation of Price - Turnover Relation
turnover in tons AzA/a
35
30
25
20
15
10
5
0
0
0,5
1
1,5
2
€ per g AzA

Quassia-MD
(0,7% Quassin)

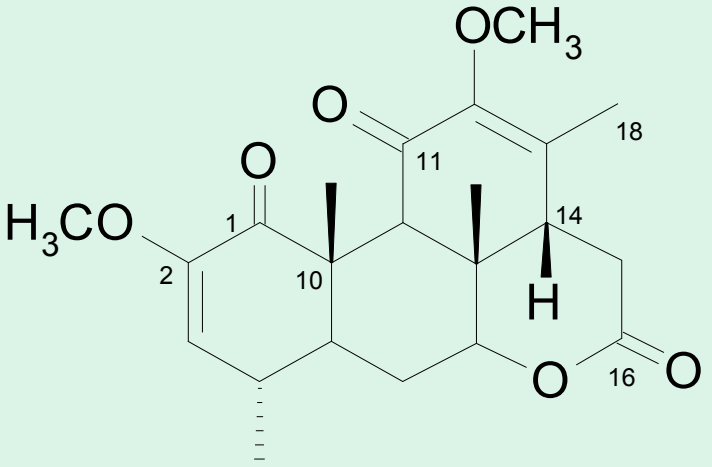
Requested for EU/91/414 Annex I

(RMS Italien; SANCO 10472)

Bitterwood: chemical structure of lead compounds



Neoquassin



Quassin

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Efficacy against the Apple saw fly

Hoplocampa testudinea **(Ludger Linnenmannstöns)**

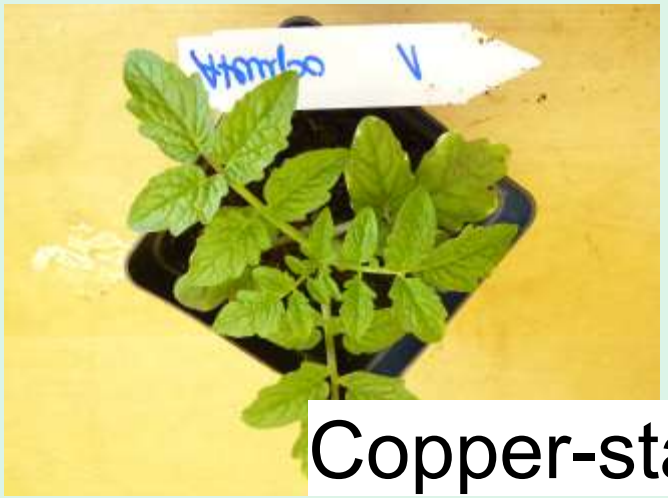
1. **Untreated**
2. **Quassia** (3,6 g/l Quassia), 1,5 l/ha per m of crown hight, treatment at 14.05.
3. **Quassia + NeemAzal T/S** (3,6 g/l Quassia), both 1,5 l/ha per m of crown hight, application at 14.05.
4. **NeemAzal T/S**, 1,5 l/ha per m of crown hight, application 14.05.

Observation 30.05.2000

Variety:	**Roter Boskoop**	**Topaz**
Untreated	28,5 % infestation	53,0 %
Quassia: Efficacy %	91,1	94,8
Quassia + NeemAzal-T/S: Efficacy %	91,1	95,7
NeemAzal-T/S: Efficacy %	69,3	80,2

Glycyrrhiza glabra leave extract

Late Blight Control on Tomatoes



Thank you for your kind attention!

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