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Practice Oriented Results on Use and Production of Plant Extracts and Pheromones in Integrated and Biological Pest Control

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Modern Developments of Methods for the Control of Plant Pests and Ectoparasites in Agriculture

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On behalf of different reasons research for the development of new active ingredients for the control of insect pests is going on in chemical companies, universities, research institutions an small enterprises. In addition to commercial reasons the development of resistancies of the pest insects against relatively simple synthetic substances is a driving force for new developments. Furthermore the minimisation of toxicological and ecotoxicological side-effects for pest control products is a declared aim. Methods for biological pest control have the additional advantage, that their production is achieved by a minimal input of fossile energy and that the products usually are highly specific, easily biodegradable and produced with minimal risk to environmental pollution.

In many countries integrated methods for pest and resistancy management are state of the art. The ongoing discussions concerning the quality of agricultural production are leading to higher levels of consciousness in the public, increased awareness on the usage of synthetic chemicals vs. biological means and marketing conceptions which favour agricultural produce which is produced according to IPM or organic farming guidelines. In this connection the concern of the consumer is not the philosophy behind the production but the demand for produce free of residues.

Although the legal requirements for the official registration of products for biological pest control are the same or even higher (due to the complexity of the nature of the biological products) as for synthetic chemicals, their specificity is usually much higher; consequently their potential market volume and hence their economic profit chance is small. Due to the enormous – and increasing - expenditure for the development and registration of pest control agents (currently several hundred million of DM) only concerted efforts of environmentally and socioeconomically concerned groups may lead to new products in addition to political decisions.

In addition to the obviously favourable use of beneficial insects in agriculture the intensification of the application of pheromones, different plant extracts, products of microbiological origin and different mineral products seems possible.

The potential uses of pheromones in the frame-work of monitoring, mass trapping or mating disruption strategies are obvious and need further introduction into the practice. Due to the very high specificity of the pheromones this seems to be a laborious and consequently expensive process.

Research for active substances originating from or containing microorganisms (including viruses) is going on world-wide. In the European Union more than 20 microbiological products for the control of pest insects or fungi and plant diseases are under practical evaluation.

Mineral products are still widely used but may in specific cases exhibit undesired side-effects especially with respect to beneficial insects.

In comparison to vegetable oils some extracts of different parts of plants may have a highly specific potential for pest control. Among these, extracts of Chrysanthemum flowers (containing pyrethrins), Bitterwood (containing quassin) and Neem seeds (containing azadirachtins) are used traditionally and seem to have a practical potential in future.

The official registration procedure and the legislative control bodies take care that the properties of commercial products are within the accepted limits. It is obvious that uncontrolled and usually non-standardised traditional extracts of the above mentioned plants may vary considerably in quality. This may lead to severe failures of the application in practice and consequently serious economic losses of farmers as well as damage to the environment. For this reason the standardisation of means for biological is indispensable for modern products for biological plant protection.

POSSIBLE USES OF NEEM – TRADITIONAL METHODS OF INDIA AND MODERN METHODS OF PEST CONTROL

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The Neem tree

Leaves, seed kernels, bark and roots of the tropical Neem tree *Azadirachta indica* A. Juss are used in India since times immemorial for curing many diseases. In a holistic perception the protection of plants and animals against diseases and illness is a medical issue as well. The leaves and especially the seed kernels of the Neem tree and their extracts have been used for the control of various insect pests in India. Our research has combined the experience of the thousands years old Indian experience and modern demands for plant protection products. The result of our development is the Azadirachtin concentrate "NeemAzal™" and its formulations like NeemAzal™-T/S.

What is NeemAzal-T/S?

NeemAzal-T/S is a formulation of the highly concentrated active ingredients of the Neem tree, namely the Azadirachtins. This concentrate – named "NeemAzal" – contains in an average 34% AzadirachtinA, about 20% other Azadirachtins and 46% of inert ingredients like lipids, oligosaccharides, hydrate water. NeemAzal may for example be formulated with the help of surfactants (produced from renewable resources) and/or plant oils to obtain a concentrate with sufficient shelf life for practical application.

Physico-chemistry and degradation

NeemAzal-formulations usually have a shelf life of more than 2 years if stored below 20°C in a dark place. They spread easily for example on leaf surfaces and show good penetration of the fur of animals. An octanol-water distribution coefficient below 10 indicates a low potential for accumulation in fatty tissue and hence in the food chain. Azadirachtins are not much adsorbed by soil and thus leach rapidly. However, the degradation is very fast, so that a risk of contamination of ground water can be excluded. In water NeemAzal is transformed very rapidly by light. After spray application to leaves and fruits AzadirachtinA is degraded rapidly with a half life of the order of very few days.

Toxicology

NeemAzal and the formulation NeemAzal-T/S has been investigated thoroughly with respect to possible toxicological impacts to mammals. Neither acute nor subchronic or chronic studies indicate the presence of important risks for humans or mammals. This is especially established with respect to carcinogenicity, teratogenicity, reproduction etc. In this connection it is important to state that these "non-toxic" properties refer only to the concentrate NeemAzal and its standardised formulation and not to other "Neem-products" since these may have considerably different compositions.

Ecotoxicology

NeemAzal-T/S has been studied carefully with respect to possible side effects on the environment. The high "No Observable Effect Concentrations" NOEC indicate an extremely low risk to aquatic organisms; this is true especially in view of the low concentrations of AzadirachtinA which are necessary for efficient applications in practice (i.e. of the order of 15 to 30 ppm AzadirachtinA in typical spraying solutions).

Beneficials are generally not influenced to a meaningful extent by NeemAzal-T/S applications - with the exception of thin skinned species (like syrphids).

Acute as well as reproduction studies with honey bees show that no adverse effects may be considered after application of NeemAzal-T/S. Studies on chicken as well as field observations do not show any significant effects with respect to birds.

Mode of action

After the treatment with NeemAzal™-T/S larvae react with feeding and moulting inhibition and mortality; adult (beetle) show feeding inhibition, infertility and to a lesser degree mortality.

As a result of this comparatively slow **„insectistatic" mode of action** of NeemAzal™-T/S a final assessment of the treatment should be done 7-10 days after application under practical conditions. The number of dead pest insects is not necessarily a good evaluation criterion. For the assessment the following criteria are appropriate: loss of leaf mass, damage to plants, formation of honey dew, crop yield, development of the pest population, positive effects on beneficials.

The success of the application with NeemAzal™-T/S depends on the progress of the pest infestation and adequate timing of the treatment.

In the case of **a** temporary **infestation and synchronous development** of pest populations one application per generation or season is generally sufficient (under European climatic conditions, usually one or two generations, for example: appearance of fundatrices of the Rosy Apple Aphid *Dysaphis plantaginea,* first adults of Elder Bush Aphid *Aphis sambuci* (Hom., Aphididae), first young larvae of Colorado Beetle *Leptinotarsa decemlineata*, beginning of flight of Cockchafers (*Melolontha* sp.)).

In case of a **permanent** infestation (several generations like Aphids, Thrips, White Flies, Spider Mites etc.) repetitive applications are required. The interval between treatments is usually 7 to 14 days and depends on climatic conditions and infestation pressure.

NeemAzal™-T/S is harmless to most beneficials - they are an important factor in the control of the remainder of the pest population. **NeemAzal™-T/S** can favourably be combined with the use of beneficials in plant protection conceptions.

Phytotoxicity information

NeemAzal™-T/S was tested with many plants under outdoor and greenhouse conditions and shows generally good plant compatibility during the warm season. The compatibility of **NeemAzal™-T/S** depends on the variety and species of plants. Some ornamental varieties react with leaf and blossom damages. Some pear varieties react with strong leaf necrosis. In the case of plant species that normally react insensitive, individual varieties can exhibit incompatibilities and it is proposed to perform sensitivity tests with a few plants or some leaves in the respective stadium of growth 3 to 5 days before treatment of larger areas.

NEEM IN BRAZIL - PLANTATIONS, EXTRACTS, RESEARCH AND UTILIZATION

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During the decade of 1930, Brazil used to extract rotenone from several vegetal species, which were used in the country and exported mainly to USA. Also pyrethrum and nicotine were extracted from *Chrysanthemum cinerariaefolium* and *Nicotiana tabaci.* This market was replaced by the commercialization of synthetic molecules and, after 1950, Brazil started to import and apply chemical products in agriculture, with their notorious consequences to vertebrates and to the environment. In Brazil, several conditions aggravate this picture, like: high costs of the products, incorrect use, low level of instruction of the growers, low surveillance by the government and climatic inadequacy of the protective clothes and consequent low use. This situation demands new methods of pest control alternative the chemical insecticides, less toxic to men, less pollutant, with lower residues, cheap and which can be produced in the farm. Changes in the profile of producers and consumers are the most recent tendencies: consumers are more concerned to the possible risks caused to health by food produced conventionally, producers are interested in less pollutant technology, besides the fact that the organic products can achieve better prices in the market and their area has considerably increased.

Neem and its products fit quite well in this expectance due to the nature of the compounds, mode of action, very low toxicity, among others. In Brazil, neem research started in IAPAR, Londrina County – PR, with the introduction of neem seeds from Philippines, in 1986. Although the neem tree is typically tropical, the material introduced from Philippines grew well and produced fruits in small quantities. The next step was to evaluate the adaptation of neem biotypes from different origin in several localities in Brazil. For this purpose, seeds were imported from Dominican Republic, Nicaragua and India (Pune) and planted in Londrina and Paranavai (Parana State), Jaboticabal (Sao Paulo State) and Brasilia (Federal District). Although Brasilia had the best conditions to grow the neem tree, the work was interrupted by difficulties in conducting the experiments. Similar development was observed among plants from different origin. The genetic variability was very high and trees with very diverse characteristics were obtained from the same material. The best development took place under the highest temperature and sandy soil, which happened in Paranavai. In all regions, significant fruit production occurred after about four years, reaching 7 to 8 kg fresh fruit/plant. Flowering goes from December to April and fruiting happens from March to May.

From these findings came the interest of growers to plant neem tree, mainly in Central Region of Brazil, where the tropical climate is more favorable. At the moment, there are near 150,000 neem trees distributed in different states, mainly: Federal District, Goias, Para, Parana, Sao Paulo and Tocantins. These plantations are young, most of them not more than three to four year old. They were established to produce seeds, seedlings, leaves, fruits and wood. However, this production still finds limitations, like lack of adapted varieties and of technologies to cultivate and harvest, high costs of labor and marked not yet clarified.

Several products start to be available in the market, like: emulsified oil, dry leaves, grounded seeds, antiseptic cream and shampoos for animal, soaps, among others. However, these products still need to have their technology of production and stability improved, need to be registered, besides recommendations provided by research on the organisms they can control, doses, methods of application, etc.

The scientific research with neem in Brazil has spread out more recently, as an answer to these needs. In IAPAR the action of neem extracts and neem oil has been studied on pests of economic importance, mainly coffee pests and also on natural enemies. Development, consumption, reproduction, mortality in different life stages and repellency were evaluated. Neem oil or leaf and fruit extracts were efficient on the following species: *Leucoptera coffeella*, *Diabrotica speciosa*, *Bemisia tabaci*, *Spodoptora frugiperda*, *S. littoralis*, *Alabama argillacea* and on the mites *Brevipalpus phoenicis*, *Phyllocoptruta oleivora*, *Tetranychus urticae* and *Polyphagotarsonemus latus.* The predator *Cycloneda sanguinea* was proved less susceptible to neem oil. Other research institutes and universities have included nim in their investigations, like: Universidade Federal de Viçosa/MG, Universidade de Goias/Goias, Embrapa Arroz e Feijão/Goias, Escola Superior de Agricultura Luiz de Queirós/SP, Universidade Estadual de Londrina /PR, Embrapa Tabuleiros Costeiros, Embrapa Milho e Sorgo/MG, Universidade Federal Rural de Pernambuco/PE, Faculdade de Ciencias Agricolas e Veterinaria de Jaboticabal -UNESP/Sao Paulo. Some of the results are here described. Neem oil caused larval mortality of *Plutella xylostella* in Brassicae (Maranhão *et al*., 1997) and reduced egg viability and caused mortality of the mite *Mononychellus tanajoa* (Gonçalves, 2000); leaf extracts caused larval mortality of *S. frugiperda* in corn (Viana *et al*., 2000); neem oil caused mortality and prevented the development of populations of *Calosobruchus maculatus* in stored beans (Oliveira *et al*., 1998); neem extracts are also being studied to control ticks and *Hematobia irritans* in cattle (H. Amorim).

IAPAR develops research with the tree, aiming to obtain plants more adapted to subtropical conditions and to test the adaptation of neem grafted on *Melia azedarach*, more adapted in subtropical conditions. Besides, evaluates azadirachtin content along plant phenology and within extracts stored under different conditions.

Brazilian experiences and tendencies till the moment indicate a great potential of production and use of neem based technologies in the country, mainly due to the wide areas with climate favorable to plant neem trees, to the interest of growers, and to the possibility to develop technology to produce and harvest neem and to industrialize products, besides the wide potential market.

PROPERTIES OF NEEMAZAL™-T/S – EXPERIENCES AND POSSIBILITIES IN BIOLOGICAL PLANT PROTECTION

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During the period 1994-2000 we have optimised the commercial formulation NeemAzal™-T/S with respect to its application by field and laboratory trials. The efficacy was tested against more 140 species of mites (*Acari)* and insects from *Coleoptera, Diptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera* and *Thysanoptera.* The results (see table) show, that NeemAzal-T/S is effective against a large variety of free feeding sucking and biting pests. In the phytotoxiticy experiments it was observed, that the formulation may be toxic to certain varieties of pear-trees and certain varieties of ornamentals in greenhouses. In order to obtain reliable results with respect to efficacy and phytotoxicity many tests have to be performed under various practical conditions.

In October 1998 we obtained the registration for the use of NeemAzal-T/S in Germany for the control of sucking insects, white flies, leaf miners and spider mites ornamentals in greenhouses and in 2000 for Rosy Apple Aphid, Colorado Potato Beetle, Winter Moth and other pest for field-application.

All results show that the application of NeemAzal-T/S is efficient with respect to the registered target pests and does not bear special risks to humans and the environment.

Due to these favourable properties work for the expansion of the registration for plant protection is in progress. Formulations which may efficiently be used for the control of human or animal ectoparasites have been developed and are currently tested.

From the various results the effects of the NeemAzal application can be summarised in the term **„Insectistatic“.** The main aspects of this mode of action are:

Phenomenon	**Timing**	**Description**	**Assessment**
Feeding inhibition	after hours	reduced food uptake	reduction of: plant damage, faeces, honey dew
Inactivity	after days to 1-2 weeks	over all reduction of: fitness, molting inhibition, starvation	mortality
Fertility reduction	after weeks (next generation)	reduction of progeny	reduction of the next population

Summary of results with NeemAzal-T/S

Index*	Species	Pest	Number of results:		
			positive	undecisive	negative
	Acari, Tetrapodili				
0	***Eriophyes rubi, E. gracilis***	Blackberry Gallmite	-	-	2
	Acari, Tetranychidae				
+ + +	***Oliconychus coffeae***	Red Spider Mite	10	-	-
+ + +	*Panonychus ulmi*	-	2	1-	-
+ + +	*Tetranychus cinnabarinus*	Spider Mite	1	-	-
+ + +	*Tetranychus urticae*	Spider Mite	15	4	1
	Coleoptera				
0	*Raphidodopalpa foveicollis*	-	2	-	-
	Coleoptera, Chrysomelidae				
+ + +	***Criocerus asparagi, C.duodec.***	-	2	-	-
+ + +	***Chrysolina varians***	-	1	-	-
+ + +	***Galerucella nymphaeae***	-	2	-	-
+ +	*Dicladispa armigera*	Rice Hispa	2	2	1
0	*Gastroidae viridula*	-	-	1	-
+ + +	*Leptinotarsa decemlineata*	Color. Pot. Beetle	26	-	1
+ + +	*Oulema melanopus*	-	1	-	-
+ + +	*Phaedon cochleariae*	-	1	-	-
0	*Phyllotreta sp.*	-	2	3	3
0	*Psylliodes od. Phyllotreta sp.*	-	-	2	-

	Coleoptera, Coccinellidae				
+ +	***Epilachna vagintioctopunctata***	Epilachna Beetle	1	-	-
+ +	*Henosepilachna vigintioctp. .*	Afr. Mel. Ladybird	3	1	1
	Coleoptera, Curculionidae				
0	*Anthonomus pomorum*	-	1	-	1
-	*Ceuthorrhynchus assimilis, C.napi*	-	-	-	4
-	*Curculio nucum*	-	-	-	1
0	***Coenorhinus aeguatus***	-	-	1	-
+	*Hylobius abietis*	-	2	-	-
0	*Otiorhynchus sulcatus*	-	1	1	-
0	*Phyllobius sp.*	-	-	-	1
0	*Phynchites bachus*	-	-	1	-
	Coleoptera, Nitidulidae				
0	*Meligethes aeneus*	-	-	-	1
	Coleoptera, Scarabaeidae				
+ + +	*Melolontha hippocastani*	Cockchafer	8	2	2
+ + +	*Melolontha melolontha*	Cockchafer	6	-	1

Index*	Species	Pest	Number of results:		
			positive	undecisive	negative
	Heteroptera, Pentatomidae				
0	***Antestiopsis orbitalis***	*Coffee Bug*	-	1	-

	Heteroptera,Miridae				
0	*Lygus pabulinus*	-	8	1	2
0	*Plesiocoris rugicollis*	-	1	1	-
	Heteroptera,Coreidae				
0	*Amblypelta lutescens*	Banana-spotting	1	-	-
	Diptera				
+ + +	***Agromyzidae***	-	2		
+ + +	***Sciaridae***	-	5	3	-
	Diptera, Agromyzidae				
+ + +	***Liriomyza huidrobrensis, L. trifolii***	Leaf Miners	8	1	1
+ + +	*Phytomyza sp.*	Leaf Miner	1	-	-
+ +	*Napomyza gymnostoma*	Leaf Miner	1	-	-
	Diptera, Anthomyiidae				
0	***Delia brassicae, D. floralis***	Cabbage Maggot	2	1	2
	Diptera, Psilidae				
0	***Psila rosae***	Carrot Fly	-	-	1
	Diptera, Cecidomyiidae				
0	***Contarinia tritici***	-	-	-	1
0	*Dasineura brassicae, D. mali*	-	1	-	1
0	*Orseolia oryzae*	Rice Gall Midge	1	-	-
	Diptera, Muscidae				
0	***Musca domestica***	-	1	-	-
	Diptera, Trypetidae				

0	*Platyparea poeciloptera*	-	-	-	1
0	*Rhagoletis cerasi*	Cherry Fly	2	4	-
	Homoptera				
+ +	*Coccidae*	Mealy Bug	2	-	-
	Homoptera, Adelgidae				
0	*Pineus pini/orientalis*	-	-	1	-
0	*Dreyfusia nordmannianae*	-	1	-	-
	Homoptera, Aleyrodidae				
+ + +	*Aleurocanthus spiniferus*	White Fly	1	-	-
+ + +	*Aleurothrixus floccosus*	White Fly	-	1	-
+ + +	*Bemisia tabaci*	Cotton White Fly	12	4	-
+ + +	*Dialeurodes kirkaldyi*	White Fly	1	-	-
+ + +	*Trialeurodes vaporariorum*	White Fly	20	7	1

Index*	Species	Pest	Number of results:		
			positive	undecisive	negative
	Homoptera, Aphididae				
+ + +	*Acyrthosiphon pisum, A. scariolae*	Green Pea Aphid	2	-	2
+	*Acyrthosiphon scariolae*	Salad Aphid	-	-	2

+ + +	*Aphis fabae*	-	7	-	1
+ + +	*Aphis gossypii*	Cotton Aphid	11	8	3
+ + +	***Aphis nasturtii***	-	2	-	-
0	*Aphis pomi*	Green Apple Aphid	3	2	2
+ + +	*Aphis sambuci*	Elder Bush Aphid	5	1	1
+ + +	*Aulacorthum circumflexum*	-	1	-	-
+ + +	*Aulacorthum solani*	-	4	-	1
+	*Brachycaudus helichrysi*	-	1	2	-
+ + +9	*Brevicoryne brassicae*	Cabbage Aphid	4	11	2
+	*Cavariella aegopodii*	-	2	-	2
+	*Cryptomyzus ribis*	-	1	-	-
0	*Drepanosiphum platanoides*	-	1	-	-
+ + +	*Dysaphis devecta*	-	3	1	-
+ + +	***Dysaphis plantaginea***	Rosy Apple Aphid	41	-	1
+ +	*Dysaphis pyri*	-	1	-	-
0	*Hyalopterus pruni*	-	-	1	-
+ + +	*Macrosiphoniella sanborni*	Black Aphid	2	1	-
+ + +	*Macrosiphum euphorbiae*	-	7	1	-
+ + +	*Macrosiphum rosae*	Green Rose Aphid	5	-	1
0	*Macrosiphum rosaeformis*	Aphid	1	-	-
+ + +	*Megoura viciae*	Vetch Aphid	2	-	-
0	***Metopolophium dirhodum***	Rosy Grain Aphid	1	-	-
0	*Myzus nicotianae*	Aphid	1	-	-
+ + +	*Myzus persicae*	-	8	-	-

0	*Nasonovia ribi snigri*	-	-	2	6
+ +	*Phorodon humuli*	Hop Aphid	4	-	1
0	*Rhopalosiphum insertum*	-	1	1	4
	Homoptera, Ortheziidae				
0	*Orthezia tillanssidae*	-	1	-	-
	Homoptera, Chaitophoridae				
0	*Chaitophorus capreae*	-	1	-	-
	Homoptera, Eriosomatidae				
0	*Eriosoma lanigerum*	-	-	-	1
	Homoptera, Cicadoidae				
+ +	*Amrasca biguttula*	Leaf Hopper	9	2	2
+ +	*Empoasca flavescens (vitis)*	Grape Leaf Hopper	-	1	1
+ +	*Idiocerus niveosparsus*	Mango Hopper	1	-	-

Index*	Species	Pest	Number of results:		
			positive	undecisive	negative
	Homoptera, Cicadellidae				
+ +	*Eupterus melissae*	Leaf Hopper	1	-	-
+ +	*Nephotettix virescens*	Green Leaf Hopper	2	2	-
0	*Nilaparvata lugens*	Leaf Hopper	-	1	-
0	*Schapoideus titanus*	Leaf Hopper	1	-	-
0	*Idiocerus niveosparsus*	Leaf Hopper	1	-	-
+ +	*Sogatella fucifera*	White Plant Hopper	1	-	-

	Homoptera, Coccidae				
+	*Coccus hesperidium*	-	-	2	-
+	*Neopulvinaria imeretina*	-	1	-	-
	Homoptera, Delphacidae				
+ +	*Nilaparvata lugens*	Brown Plant Hopper	6	1	-
	Homoptera, Diaspididae				
0	*Lepidosaphes ulmi*	-	-	1	-
	Homoptera, Lachnidae				
0	*Eulachnus agilis*	-	-	-	1
0	*Schizolachnus obscurus*	-	-	1	-
	Homoptera, Phylloxeridaea				
+ + +	*Dactulosphaera vitifoliae*	-	2	-	-
	Homoptera, Pseudo-coccidae				
0	*Planococcus citri*	Citrus Mealy Bug	-	1	-
0	*Planococcus lilacinus*	Mealy Bug	-	2	-
0	*Pseudococcus longispinus*	Mealy Bug	-	1	-
	Homoptera, Psyllidae				
0	*Agonoscena targionii*	-	1	-	-
+ + +	*Psylla pyri*	-	1	-	-
	Hymenoptera, Diprionidae				
0	*Diprion sp.*	-	1	-	-
	Hymenoptera, Tenthre-dinidae				
0	*Hoplocampa testudinea*	-	2	2	1
	Lepidoptera				

0	*Achaea janata*	Rose L. Caterpillar	1	-	-
0	*Ascotis selenaria*	Giant Looper	-	-	1
0	*Hellula sp.*	Cab. Head Borer	2	-	-
0	*Hymenia recurvalis*	Leaf Caterpillar	1	-	-
0	*Leucinodes orbonalis*	Shoot&Fruit Borer	2	1	4
	Lepidoptera, Arctiidae				
+ + +	*Hyphantria cunea*	Fall Webworm	2	-	-
	Lepidoptera, Gelechiidae				
0	*Phthorimaea operculella*	Potato Tuber Moth	-	1	-

Index*	Species	Pest	Number of results:		
			positive	undecisive	negative
	Lepidoptera, Geometridae				
+ + +	*Operophthera brumata*	Winter Moth	17	2	-
+ + +	*Bupalus piniarus*	Pine Looper	1	-	-
	Lepidoptera, Gracillariidae				
+ + +	*Phyllocnistis citrella*	-	-	50	-
+ + +	*Cameraria ohridella*	-	2	-	-
+ + +	*Lithocolletis leucographelle*	-	2	1	-
	Lepidoptera, Lasio-campidae				
0	*Dendrolimus pini*	-	2	-	-
	Lepidoptera, Lymantriidae				

+ + +	*Euproctis chrysorrhoea*	Brown Tail Moth	-	-	1
+ + +	*Lymantria dispar.*	Gypsy Moth	3	-	-
+ + +	*Lymantria monacha*	Nun Moth	2	-	-
	Lepidoptera, Lyonetiidae				
+ + +	*Leucoptera malifoliella*	Apple Leaf Miner-	-	1	-
	Lepidoptera, Noctuidae				
+ +	*Earias vittella*	Fruit & Shoot Borer	1	-	-
+ +	*Heliothis armigera*	„Amer." Bullworm	15	4	-
+ + +	*Mamestra brassicae*	Cabbage. Army Worm	11	2	-
+ +	*Mythimna albistigma*	Cutworm	1	-	-
+ +	*Spodoptera littoralis*	Eg. Cott. Leafworm	1	-	-
+	*Spodoptera litura*	Leaf Catapillar	4	5	-
	Lepidoptera, Pieridae				
+ + +	*Eurema blanda*	-	1	-	-
+ + +	*Pieris brassicae, P. rapae*	Cabbage. Butterfly	6	-	3
	Lepidoptera, Pyralidae				
0	*Cnaphalocrocis medinalis*	Rice Leaf Folder	3	5	-
0	*Diaphania hyalinata*	-	-	-	1
0	*Eurema blanda*	-	1	-	-
0	*Evergestis forficalis*	Garden Pebble Moth	1	-	-
0	*Leucinodes orbonalis*	Shoot&Fruit Borer	2	-	-
0	*Scirpophaga incertulus*	Yellow Stem Borer	2	-	-
-	*Tryporiza incertulus*	Rice Stem Borer	1	6	5
	Lepidoptera, Tineidae				

+ +	*Tineola bisselliella*	Cloth Moth	3	-	-
	Lepidoptera, Thaumetopoeidae				
+ + +	*Thaumetopoea pityocampa*	-	2	-	-
+ + +	*Thaumetopoea processionea*	Brown Tail Moth	8	2	-

Index*	Species	Pest	Number of results:		
			positive	undecisive	negative
	Lepidoptera, Tortricidae				
+ +	*Adoxophyes orana*	Sum. Tortrix Moth	4	-	1
0	***Cydia leucostoma***	Tea Flushworm	1	-	-
-	*Cydia pomonella*	Codling Moth	-	-	2
0	*Eupoecilia ambiguella*	Grape Berry Moth	-	1	1
0	*Lobesia botrana*	Europ. Grape Moth	-	2	4
0	*Pandemis heperana*	Sommer Fruit Moth	1	-	-
+ + +	*Tortrix viridana*	Green Oak Moth	-	1	-
	Lepidoptera, Yponomeutidae				
+ + +	*Plutella xylostella*	DBM	14	6	-
+ + +	***Yponomeuta malinellus, Yponomeuta padellus***		7	-	-
	Thysanoptera, Thripidae				
+ + +	*Chloethrips oryzae*	Thrips	-	1	-
+ + +	*Frankliniella occidentalis*	Thrips	12	4	1
+ + +	***Parthinothrips dracaenae***	Thrips	-	1	-
+ + +	*Scirtothrips sp.*	Tea Thrips	1	-	-
+ + +	*Taeniothrips sp.*	Tea Thrips	1	-	-

+ + +	*Thrips tabaci, T. meridionalis*	Thrips	3	5	2

***Index:** **+ + +**: efficient control established; **+ +**: efficient control possible;
+: control may be possible after optimisation of the method of application;
0 : undecided, further tests necessary;
- : efficient control not possible

Efficacies depend on: i). the time of application. ii). the concentration, and iii). the sensitivity of the target insect with respect to the different effects of the mode of action. Hence the optimisation of the application method of NeemAzal-T/S is decisive for the efficacy and the certainty of the success of the application in practice.

Taking into account the short half life of the active ingredient it seems useful to define operationally two groups of infestation:

1. batchwise appearance of the pest: with a good timing one application is sufficient for the control of one generation of the pest (like for: colorado potato beetle (1. to 2. larval instar); cockshafer (ratio male : female adults ~ 1 : 1); rosy apple aphid (appearance of fundatrices);

2. persistent infestation: at the presence of young developmental stages the application should be repeated at intervals of 1 - 3 weeks (like: thrips, white fly, or green rose aphid).

The results of practical applications show that application methods can be worked out which permit an efficient control of many different pests. These efforts seem to be worth while in order to find new solutions of pest control for biological as well as for integrated farming with a toxicologically and ecotoxicologically safe product like NeemAzal-T/S.

Phytotoxicity information

NeemAzal-T/**S** was tested with many plants under outdoor and greenhouse conditions and shows generally good plant compatibility during the warm season. The compatibility of NeemAzal-T/S depends on the variety and species of plant. Some ornamental varieties react with leaf and blossom damages. Some pear varieties react with strong leaf necrosis already from spray drift. It can not be excluded that damage can occur in cases of plants with known good compatibility.

In **ornamentals** the following plants react on NeemAzal-T/S**-**treatment with:

good leaf and blossom compatibility - *Antirrhinum majus, Acalypha hispida, Argyranthemum frutescens, Astericus,* Begonia-hybrids, *Bidens ferulifolius, Brachycome,* chrysanthema (Merced, Bronze Arola, Kory), *Celosia cristata, Convolvulus sabatius, Coreopsis* (girls eye), *Dendranthema grandiflorum, D. indicum, Diascia, Euryops chrysanthemoides, Fuchsia, F.*-hybrids, *Gazania splendens, Gerbera jamesonii, Glechoma, Helichrysum petiolare, Kalanchoe* (Boston), *Lantana*-Camara-hybrids, *Lobelia*, *L speciosa, Manettia bicolor, Mentha,* Carnations (Aristo), Slipperwort, Pelargonien, *Petunia, Pilea microphylla,* Roses (Komet), *Rudbeckia, Sanvitalia procumbens, Scaevola, Sutera, African marigold* (yellow), *Torenia fournieri, Verbena (Tapien blue)* (Sunvop (P),

good leaf compatibility - *Agerathum houstonianum, Alonsoa, Alyssum, Amaranthus,* Calceolaria hybrids, *Callistephus chinensis, Calocephalus brownii, Centaurea, Cestrum, Clarkia, Cleome, Coleus, Cosmos, Cuphea, Cynara scolymus, Dahlia, Dianthus barbatus, Dimorphoteca, Eucalyptus, Eustoma grandiflorum,* Ficus, *Felicia, Gazania, Gnaphalium, Helianthus, Heliotropium arborescens, Iresine lindenii, I. herbstii, Kochia, Lavatera, Limonium, Lotus, Lysimachia, Melampodium paludosum, Mesembryanthemum crystallinum, Nicotiana, Nigellia, Pennisetum, Penstemon, Plectranthus fruticosus, Polygonium, Portulaca, Ricinus, Salvia farinacea, Saintpaulia* (Miho io), *Senecio, Serenoa, Streptocarpus, Tanacetum, Tithonia, Trachelium,* Viola, *Veronica, Zinnia,*

blossom damages - *Begonia* semperflorens hybrids, Chrysanthema *(Deep luv), Euphorbia pulcherrima* (Peter star, Cortez), *Gerbera* (Pretty red, Sigma, Luciana), Impatiens Neu-Guinea hybrids, *Impatiens walleriana,* Pelargonium-Peltatum-hybrid, P.-Zonale-hybrids, *Solanum rantonnetti, Saintpaulia* (Miho io), *African marigold*, Verbenen (individual sorts),

leaf damages - Abutilon *hybrids*, *Cestrum, Datura, Euphorbia pulcherrima,* Impatiens Neu-Guinea hybrids, *Impatiens walleriana,* passion flower, *Solanum rantonnetti,* Roses (Papa Meilland, White Noblesse, Saphir, Ducat, Eveline, Alina, Baronesse, Lola, Black Magic, Noblesse, Roulette, Funcky Jazz, Arabia).

In **orchards** serious plant *toxicity* has been observed in the case of pear varieties ‘Conference’, ‘Alexander Lukas’, ‘Bristol Cross’, ‘Comice’, 'Guyot’, ‘HW 606’, ‘Illinois 13 bars 83 Maxi’, ‘Lectier’, ‘Trevoux’, ‘Winter dechant’.

In the case of plant species that normally react insensitive, individual varieties can exhibit incompatibilities and it is proposed to perform sensitivity tests with a few plants or some leaves in the respective stadium of growth 3 to 5 days before treatment of larger areas.

MAIN PESTS WHICH CAN POTENTIALLY BE CONTROLLED WITH TECHNIQUES BASED ON NEEM PRODUCTS IN BRAZIL

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The neem tree, *Azadirachta indica* A. Juss, contains terpenoids with repellent and deterrent properties to insects and which can also cause growth disruption, reduce fertility and fecundity and kill. They also present acaricide, nematicide and fungicide properties. The main compound is azadirachtin, which occurs in variable concentrations in aqueous and alcoholic extract prepared with leaves and fruits, oil, dry leaves and grounded fruits, fruit cake, among others. Neem effects were proved on more than 400 species of insects and many of the genera present in the literature occur in Brazil and have economic importance. However, most of the studies were run in laboratory conditions and further information need to be included before they can be recommended.

Studies run at IAPAR, in 1991 demonstrated that aqueous leaf extract added to diet caused larval mortality of *Spodoptera frugiperda* in laboratory. When sprayed on bean plants the same extract reduced egg laying of *Bemisia tabaci* in greenhouse. Azadirachtin sprayed on bean leaves reduced leaf consumption of *Diabrotica speciosa*, in a dose dependent manner. It was also shown to delay the development of *Spodoptora littoralis* (Martinez & van Emden, 1999), to reduce food intake, weigh gain and growth and to affect digestibility. It caused dose dependent mortality, crescent along *S. littoralis* development, and produced abnormalities which can be considered with mortality. Azadirachtin reduced fertility and prolonged life cycle, impairing population growth. It was more efficient when ingested than when sprayed and more lethal when administered to larvae in the final instar, producing larva-pupa intermediates (Martinez & van Emden, 2001). Neem oil was efficient against mites, which are difficult to be controlled with conventional acaricides. Oranges were sprayed with neem kernel extracts at 15 and 30% (p/v) and infested with mites. Mortalidade over 80% was observed in *Brevipalpus phoenicis*, *Phyllocoptruta oleivora*, *Tetranychus urticae*, *Polyphagotarsonemus latus*, after 24h in laboratory. In the field, neem kernel extract at 20% (p/v) caused mortality of *B. phoenicis* in citrus above 70% during 28 days, when 85% died (Meneguim & Martinez, 1998). In tests with and without choice, neem oil reduced egg laying in *Leucoptera coffeella* when coffee seedlings were sprayed (Martinez & Meneguim, 1999). Besides, neem oil caused egg mortality above 70% for those deposited on treated leaves, reducing even more the infestation potential of *L. coffeella* in coffee plantations. The action of neem oil was less evident with the predator *Cycloneda sanguinea*. Sprays of neem oil at 0,5% straight on adults did not affect survival. Larval mortality was higher but no reduction in aphids consumption was observed (Martinez & Meneguim - IAPAR).

Neem extracts are being tested on ticks and on *Hematobia Irrirans,*in cattle (H. Amorin, FCAV-UNESP/Jaboticabal-SP). Good control is obtained for both organisms when fresh leaves are added to food or leaf extracts are sprayed on the back of the animal. Spray on feces shows efficiency for *H. irritans*.

Several species of soil and airborne fungi which occur in Brazil were affected by neem extracts: *Rhizoctonia solani* (found in beans, potato, soybean) and *Fusarium oxysporium* (found in beans, soybean), *Helminthosporium nodulosum*, *Alternaria tenuis, Colletotricum.* Although neem extracts have not controlled fungi species which infect harvested fruits, they delay fruit rotting, so preventing the early contamination with fungi, like *H. nodulosum.*

Neem cake was proved efficient against nematodes. Neem prevents egg development of *Pratylenchus* sp. e *Meloydogine incognita* and reduces larval ability to penetrate the roots.

Neem and its extracts might not be taken simply as insecticides. They possess multiple effects which can be added, affecting not only the population in test but also the new generations which develop from that. By this reason, its effects deserve to be well evaluated in laboratory conditions to better understand the field results.

A most profound study on neem action on most of Brazilian pest species under laboratory and field conditions is still necessary. However we can already conclude that neem has a high potential to be used in IPM programs and in Organic Farming, by its wide range of action, multiple effects, good potential of association with biological control, special characteristics which make resistance difficult, besides the very low toxicity and quick degradation in the field.

PROCESSING OF NEEM FOR PLANT PROTECTION – SIMPLE AND SOPHISTICATED, STANDARDIZED EXTRACTS

Beate Ruch and Reinhard Wolf

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The neem tree (Azadirachta indica) is native to South Asia and grows best along the tropical belt. The areas of origin are mainly India and Myanmar. Due to the multitude of possible uses the tree has been spread throughout the world, presumably by Indian immigrants with extensive knowledge about these possibilities.

The neem tree is undemanding in view of ecological aspects (soil and water) is fast growing, has the advantage to protect areas against erosion (i.e. Sahel zone) and to halt desertification.

The global occurrence and abundance is estimated and presented in the table below.

	World	Asia/ Oceania	Africa	Caribbean/ Latin America	Industrialised Countries
Neem occurrence in million trees	60-90	27-39	31-45	5,5-6,5	approx. 0,5

The neem tree has numerous potential uses and nearly every part of the tree can be used. Details are presented in the following table.

Part of the tree	**Usage**
Seeds	Pesticides, Oil Extraction, etc.
Oil	Soap, Pesticides, Cosmetics, etc.
Cake	Plant Protection, Fertiliser, Animal Fodder
Fruits	Food, Medicine, Oil Extraction
Leaves	Medicine, Cosmetics, etc.
Part of the tree	**Usage**
Twigs	Dental Hygiene
Wood	Firewood, Construction Material, etc.

Bark	Toothpaste, Medicine
Roots	Medicine

Neem is abundant in many developing countries with low technological possibilities and financial constraints hence, farmers in their countries have to rely on simple processing techniques. The requirements for the preparation of aqueous neem seed kernel (NSK) extracts are:

- harvest or collect the fruits
- remove pulp
- dry seeds
- grind seeds
- mix aqueous extract
- sieve aqueous extract
- apply aqueous extract

Following this procedure 10 to 20 kg neem seeds per hectare treated area are needed. The advantages of aqueous extracts are that every farmer can learn how to prepare the extract (no specialists knowledge is necessary) and that no expensive machinery is involved in the production. Quite often the aqueous NSK extracts give satisfying results and the cost/benefit ratio seems acceptable but there are some disadvantages which have to be discussed.

The above mentioned requirements for the production of NSK extracts are extremely labour intensive and the collecting or harvesting of the fruits takes place at a time where other crops have the highest demands. As a result the farmer can invest less time for the crops which are necessary for his income. Furthermore the proper treatment of the neem fruits and seeds requires in fact specialist knowledge. Otherwise unwanted by-products like mycotoxins may be formed! The most important disadvantage is, that the aqueous extracts do not have a standardised content of active principles. This results in unknown quantities of active ingredients applied to the crop. This may lead to either an insufficient or an excess amount of a.i. for effective pest control. This in turn leads either to a failure of crop protection or suboptimal use of available a.i., which is in both cases a reason for increasing costs. In the worst case the farmer will lose confidence in biological pest control and return to synthetic pesticides.

There are other possibilities for biological pest control i.e. “Ready To Use” neem products such as NeemAzal-T/S. This EC formulation is made from neem seeds of controlled quality and it is produced in a resource saving technical process where most of the input is recycled. The content of active principles is monitored per batch and adjusted in the EC formulation to a standard value. The concentration of mycotoxins is monitored and controlled to a level below the threshold declared safe for food (4 µg/kg - German legislation).

A large number of studies have been performed to determine the necessary amount of NeemAzal-T/S to be applied per hectare for pest control. Because of the reliable quality of NeemAzal-T/S and the standardized concentration of a.i. the farmer can apply NeemAzal-T/S equally exactly. There will be neither losses of crop due to inadequate amounts of a.i. nor financial losses due to excess amounts of a.i. applied.

NeemAzal-T/S is an easy to use formulation: that means no additional labour is required and the farmer can concentrate on his value crops. Another advantage is the high storage stability for a minimum of 2 years without significant loss of a.i.

NeemAzal-T/S can be used in a fashion similar to other conventional pesticides with the benefit of environmental safety.

QUALITY CONTROL OF NEEM MATERIAL

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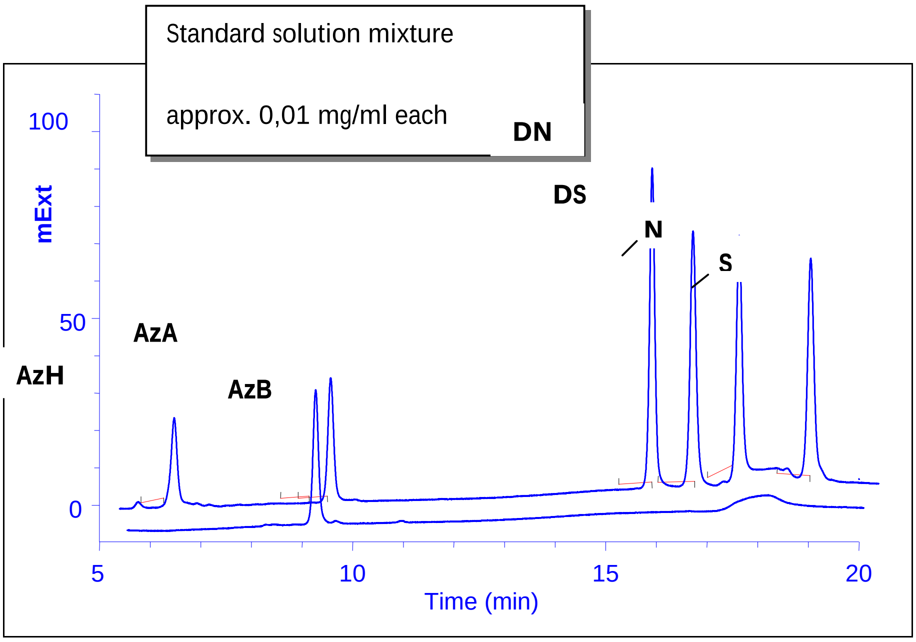
For pest control purposes the most interesting part of the Neem tree is neem seed material which comprises the insecticial ingredient Azadirachtin A. Furthermore there are other Azadirachtins (Azadirachtin B, Azadirachtin H, etc.) as well as Nimbines and Salannins. Depending on the quality there are 2 to 9 grams acitve ingredients per kilogram seed material.

In this context quality control of neem material is mainly the analysis of active ingredients of the Neem seed kernels, Neemoil and Neem formulations.

The analytical method is the high performance liquid chromatography – HPLC. Best results are obtained with a UV detection of 214 nm, columns filled with reversed phase C18 or C12 material, and eluents which consits of H_2O mixtures with CH_3OH or CH_3CN. A HPLC chromatogram of available Neem standard solutions is presented in the following diagram.



AzH: Azadirachtin H; **AzB**: Azadirachtin B; **AzA**: Azadirachtin A

DN: Desacetyl-Nimbin; **DS**: Desacetyl-Salannin; **N**: Nimbin; **S**: Salannin

The most important Neem ingredient is Azadirachtin A. It is the major compound for insect control for plant protection purposes as well as for control of human and animal ectoparasites.

For comparison of analytical results the GTZ Pesicide Service Project (Germany) initiated a collaboration of Neem experts to develop a standard method for sample preparation and analysis. The results are published as CIPAC method no. 4042 **“High performance liquid chromatographic method for the analysis of Azadirachtin in Neem kernels, Neemoil and formulated prodcuts”.**

Experimental aspects of this method:

Generally the determination of Azadirachtin A in an analysis solution should be performed as soon as possible after sample preparation. If this is not possible the sample has to be stored in a refrigeratior with T < -15°C. Each sample has to be analysed in duplicate and at least two independent analysis solutions from each sample have to be prepared.

Sample preparation

Neemoil Approx. 250 mg of Neemoil plus 10 drops of Tween 85 are dissolved in a 50 ml flask with the chromatographic eluent

HPLC analysis

Neem formulation Approx. 50 mg of formulated product is dissolved in the HPLC eluent and filled up to the final volume of 100 ml

HPLC analysis

Neem kernels1) Weight loss of Neem kernels by drying

A container with approx. 5 g kernels (shell has to be removed) has to be placed in an oven with 103 ± 2 °C for 17 ± 1 h. After drying the material has to be cooled down in a desiccator for 30-45 min. The rel. humidity in the lab should not exceed 70%.

2) Extraction of Azadirachtin A of Neem kernels

Approx. 3 g kernels in 30 ml methanol have to be homogenised with a tissue mixer for 3 minutes. After filtration of the extract this procedure has to be repeated twice. The filtrates have to be combined in a 100 ml flask and filled up to the mark with methanol. An aliquot of this solution is diluted in HPLC eluent (1:10 v/v)

HPLC analysis

Calibration

At least 5 standard solution should cover a concentration range of 1 to 50 µg/ml for setting up a calibration curve. For quantification of the Azadirachtin A content the mean response factors (Azadirachtin A content / peak area of the calibrant) or the regression parameters of the calibration curve ($y = ax + b$) have to be taken into account.

Calculation of the Azadirachtin A (AzA) content

Neem kernels:

$$AzA_{Sample} = \frac{AzA_{dil} * V * DF * 100}{W * (100 - MC)}$$

AzASample	AzA content of the sample [mg/g dry matter]
AzAdil	AzA content in the dilution [mg/ml]
V	total volume of extract
DF	dilution factor
W	fresh weight of the sample
MC	moisture content – mean weight loss on drying [%]

The factor (100-MC) has to be neglected for calculations of Azadirachtin A in Neemoils and Neem formulations.

NeemAzal-T/S – Estimation of residue data based on the analytics of the leading compound Azadirachtin A

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Registration of a plant protective agent in Germany requires submission of reports for different subjects, e.g. toxicology, efficacy.

Residue behaviour of plant protective agents is a very important aspect for registration purposes. Azadirachtin A is established as a leading compound for analytical purposes in the active ingredient NeemAzal and its formulation NeemAzal-T/S.

Before starting with residue studies a lot of preliminary work has to be done. First a method for a special problem (i.e. analysis of Azadirachtin A in soil) has to be developed. This includes the extraction of Azadirachtin A from the matrix, purification of the extract and finally HPLC-analysis. This development is followed by the validation of the method where the limit of detection and quantification, the recovery rate, accuray and precision as well as specifity and selectivity have to be determined.

Residue studies in water are important for the control of the concentrations of NeemAzal and NeemAzal-T/S in tests of toxicological and ecotoxicological relevance. It is important to keep in mind, that the half-life-time of Azadirachtin A in water is dependent strongly on the pH.

pH	Temperature [°C]	half-life time [d]
4	20	49,9
7	20	19,5
8	20	4,4

Method for residue analysis in water

An extraction procedure is not necessary. The water samples have to be concentrated, dependent on the expected concentration of Azadirachtin A in the sample. It is strongly recommended to perform a solid phase extraction for the concentration, because the Azadirachtin A will degrade during evoparation of the water at higher temperatures.

Residue analysis in soil is an important tool for monitoring and controlling of the concentrations of NeemAzal and NeemAzal-T/S in tests of ecotoxicological and environmental relevance. These tests are adsorption/desorption studies, leaching activity, degradation in soil and side effects on soil micro flora (earthworms).

Method for residue analysis in soil

Approx. 50 g soil has to be extracted with 50 ml CH_3OH followed by filtration and washing. Afterwards a solid phase extraction for purification has to performed. It may be necessary to make use of different solid phase sorbents to obtain a sufficient purificated extract.

Residue analysis in plant material is necessary for the evaluation of MRL values (Maximum Residue Levels) and ADI values (Acceptable Daily Intake) by the authorities. These values are important for the evaluation of waiting periods after the last treatment of plant material with NeemAzal-T/S. The aim of this work is protection of the consumer.

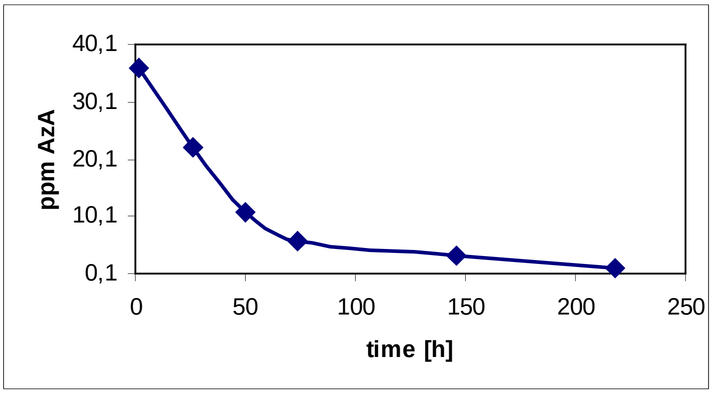
Method for residue analysis in plant material

First step is an extraction procedure where the plant material has to be homogenized thoroughly. The amount of plant material and the choice of the solvent is dependet on the plant matrix. At least two solid phase extraction are necessary (polar and nonpolar sorbents). Up to now it is not possible to present a general method for extraction and sample preparation of different plant materials.

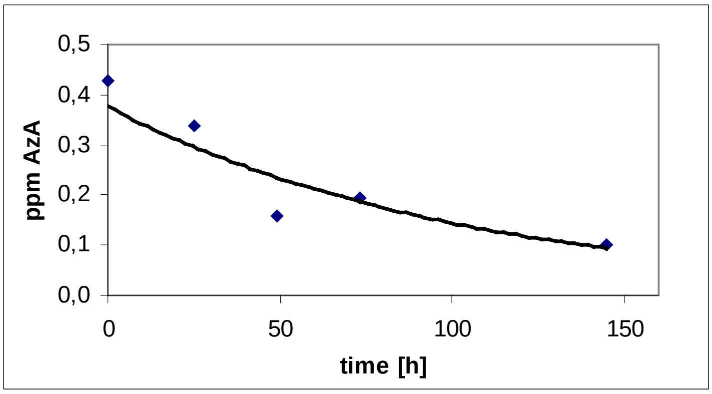
Results of residue studies have shown that there is a possiblily to divide plant material in two groups:

- leafy vegetables (lettuce, spinach, etc.)
- fruity vegetables and fruits (tomatoes, apples, etc.)

A comparison of the decrease of Azadirachtin A on/in tomato leaves (representative for leafy vegetables) and tomatoes is presented in the following diagrams.



Azadirachtin A on/in tomato leaves after treatment with the tenfold recommended concentration (5% NeemAzal-T/S)



Azadirachtin A on/in tomateo after treatment with the tenfold recommended concentration

(5% NeemAzal-T/S)

Aside from the different half-life-times in tomato leaves and tomatoes the concentration of Azadirachtin A directly after treatment is different as well. Other residue studies show the same tendency - the leafy vegetables show a higher initial concentration of Azadirachtin A (approx. 3 mg Azadirachtin A per kg) whereas the fruity vegetables and fruits show an initial concentration of approx. 0,1 mg Azadirachtin A per kg.

Those results help us to propose waiting periods for the estimation of residues in plant material. On basis of the “Diätverordnung” which is the strictest limit of the German authorities regarding residues in food (it demands that less than 0,01 mg residue per kg is existing) we have to wait for 8-9 days after treatment with the consum of leafy vegetabels and 9-12 days for fruity vegetables and fruits respectively.

SEMIOCHEMICALS IN AGRICULTURE: THE USE OF PHEROMONES AND ALELOCHEMICS IN PLANT SYSTEMIC RESISTANCE

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1. Introduction: Chemical Mediators in the Organic Agricultural context

Besides the undeniable knowledge and yield (per unit area) achievements of Agronomic Science in the 20th Century, its progresses have brought with them a paradoxical increase in both the incidence and number de species of pests e diseases on world crops (Altieri, 1995; Paschoal, 1994 & 1995). Parallel to this, a marked decrease on food quality was recorded, both due to losses of nutritional qualities as to food and environmental contamination by agrochemicals, generating the so-called "agricologenic diseases", *i.e.*, primarily caused by agricultural practices and techniques (Deffune, 2000; Hodges & Scofield, 1983).

The not-so-recent advances in the research on chemical mediators (Nordlund *et al*., 1981) of decisive importance on both intra and interspecific (*e.g.*; between plants, plant-microbe and plant-insect) interactions, show that while it is surely impossible to totally eradicate a micro-organism, worm or arthropod which happen to be adapted to favourable conditions in agricultural systems, it seems well achievable to develop strategies which can "fool" or deviate these highly specialized and biologically programmed organisms (in relation to their respective food chains and webs) from crops which can be, on their turn, selected, strengthened and in a large extent immunized against economic levels of damage. In this case, a systemic strategy or holistic approach must especially combine the factors (among others) of soil fertility management with the chemical and dynamic mediation mechanisms between organisms in the agro-ecosystems, as suggested by the very founders of Organic Agriculture (Steiner, 2000/1924; Howard, 1940a&b; Chaboussou, 1972, 1980 & 1985).

2. Plant Resistance Mechanisms

The importance of acquired and induced resistance mechanisms in plants has been known for quite a long time, since the works of Chester (1933) and Gaumann (1946).

The natural protection of plants against pathogenic agents and herbivores (*e.g*. insects, mites, molluscs) is given by several mechanisms. It is partly based on a variety of constitutional barriers already present in the plant tissues prior to any attack or infection. The combined effect of all these barriers is called Constitutional Resistance. Additionally, through stress or inoculation, plants can activate or be stimulated to produce a variety of biochemical protection mechanisms called Acquired or Induced Resistance Mechanisms. As these stimuli or signals can translocate or communicate from the primary stress or inoculation loci, toward reasonably distant tissues, promoting systemic defence reactions, the term "Systemic" has been added both to Acquired and Induced Resistance (Sticher *et al*., 1997).

These last two mechanisms, SAR and ISR, are distinguished from each other by the nature of either the induction method or the inducing agent (or elicitor) which provokes, or, to be more precise, evokes the reaction, as follows:

1. When the induction is unintentional and/or the elicitor is a pathogenic agent or parasite, the reaction is called Systemic Acquired Resistance (SAR).

2. When the induction is intentionally promoted and/or the elicitor is a beneficial, symbiotic or neutral agent (*i.e.*, neither a pathogenic nor a parasitic organism), or it is abiotic (*e.g.* mineral, apneumonic, dead), the reaction is called Induced Systemic Resistance (ISR).

Since both mechanisms depend on signal cascades of the biochemical or biodynamic type (for being properties of living, autopoietic or self-regulated organisms), which cannot be isolated from the plant's general metabolism, it can be deduced that both SAR and ISR are linked to other induced metabolic adaptations in plants, which can effectively influence parameters like growth, biomass, reproduction, yield and quality (Abele, 1973; Spiess, 1978; Samaras, 1977; Margulis, 1995; Koepf, 1993 & Koepf *et al*., 1996).

3. Chemical Mediators, Applied Allelopathy and Metabolic-Dynamic Induction

This induction of different metabolic adaptations in plants, animals and microbes is regulated by chemical mediators or "**Mediochemicals**" which are classified according to their functional role (Nordlund *et al*., 1981; Deffune, 2000a), as shown in Figure 1.

Apneumones can constitute a separate group of compounds, independent of allelochemics, which would include abiotic elicitors and biodynamic preparations (Nordlund, 1981; Deffune, 2000a).

	Class	Sub-Class	Functional Category
CHEMICAL MEDIATORS	**1. Hormones**: Intra-individual chemical messengers (produced inside a single organism)	**1.1. Plant**:	Auxins, Cytokinins, Gibberelins, Abscisic Acid, etc
		1.2. Animal:	Ecdysone, Juvenile and others not present in humans
		1.3. Human:	Adrenalin, Insulin, Thyroxin, ADH, TSH, FSH, LH, etc
	2. Semiochemicals: Inter-individual chemical messengers, active between different organisms	**2.1. Pheromones**: Intraspecific Interactions	**2.1.1. Sexual**
			2.1.2. Alarm
			2.1.3. Epideictic (spacing)
			2.1.4. Attractive, Aggregating, etc
		2.2. Allelochemics: Interspecific Interactions Between Living Organisms	**2.2.1. Allomones**: Beneficial for Emitter Detrimental for Receiver
			2.2.2. Kairomones: Beneficial for Receiver Detrimental for Emitter
			2.2.3. Synomones: Beneficial for Both
	Effects produced by **Non-Living Substances** on Organisms	→	**2.2.4. Apneumones**: Stimulatory or Inhibitory

Figure 1. Schematic classification of Chemical Mediators.

According to Rice (1984), the antagonistic allelopathic agents or **Allomones** (item 2.2.1 in Figure 1) functionally subdivide themselves in four classes:

3.1. **Antibiotic**: a chemical produced by a micro-organism and effective against another micro-organism.

3.2. **Koline**: chemical produced by a higher plant and effective against another higher plant.

3.3. **Marasmine**: a compound produced by a micro-organism, which is active against a higher plant, term proposed by Gaumann (1946).

3.4. **Phytoncide**: proposed by Waksman (1952) for an agent produced by a higher plant, which is effective against a micro-organism. Nevertheless the term can be extended to plant agents with detrimental effects on arthropods and herbivores in general.

This classification sheds new light on the so-called allelopathic interactions in agroecosystems, offering new forms of crop management (*e.g.*; companion plants and weed suppressors) and the development of techniques which constitute the new face of Applied Allelopathy (Rice, 1995; Deffune, 2000). Phytoalexins, or plant chemical defence substances are today understood in the wider context of Plant Systemic Resistance and Semiochemicals (Deverall, 1977; Nordlund, 1981). According to the functional schematic classification of Semiochemicals (mediators chemical between different individual organisms), phytoalexins are specifically defined as Phytoncidal Allomones, *i.e.*, Allelochemicals (interspecific semiochemicals) with beneficial effects for the emitting plants and detrimental for phytopathogenic micro-organisms (Waksman, 1952; Rice, 1984; Deffune, 2000). Thus, natural pesticides like Neem (*Azadirachta indica*) extracts can be classified as insecticidal Phytoncides (Allomones) or even as Apneumones.

From the knowledge of the forms of action of different chemical mediators, one can study the possible mechanisms and applications involved, which can be due to mass-action dependant biochemical reactions (in relation to the participating constituent elements), or to dynamic or energetic "pulses" evoked by ultra-high dilutions (Endler & Schulte, 1994).

4. Processes involved in ISR and SAR

The respected French researcher Francis Chaboussou (1980 & 1985) ha already anticipated the ISR and SAR mechanisms as he suggested that conventionally cultivated plants were sick because agrochemicals (pesticides and fertilizers) would deviate secondary metabolic processes from the balanced production of defence structures and substances, generating an excess of free amino-acids and soluble nutrients (especially nitrogen). This unbalance would leave plant cells excessively turgid and weak, promoting the proliferation of pests and diseases. On the other hand, Chaboussou also understood that the elimination of positive natural stress factors - for example, contact with a soil rich in organic matter and microbes, would leave plants less resistant to parasite attacks. Contrarily, he mentioned that traditional treatments like Bordeaux Mixture seemed to stimulate a natural reaction of the plant and he's foreseen in this non-antagonistic approach of plant health a true "agronomic revolution" (Chaboussou, 1980 & 1985).

In this sense, research has accumulated evidences on the effectiveness of apneumonic substances containing eliciting agents for SAR (*e.g.*, alkaloids, flavonoids, terpenoids, cumarins, sulphites, glucosídios, tannins, purines, organic fatty acids) frequently present in compost and organic manures in general, as well as in allelochemical and biodynamic preparations (Doubrava *et al*., 1988; Koepf, 1993). The ubiquitous jasmonates, for example, which act in very low concentrations both as allomones (*i.e.*, antagonistic metabolites) against insects and inducers of SAR, are biosynthesized (probably through lipoxygenase action) from linolenic acid, which is present in the chloroplasts' thylakoids (the grana and stroma lamellae) of most plant species (Sticher *et al*., 1997; Salisbury & Ross, 1992).

Therefore, both the action and elicitation of phytoalexins are intimately related to wider SAR and ISR mechanisms, as shown in the review by Sticher *et al.* (1997) on their general effectiveness and on the biochemical pathways through which fatty acids (*e.g.*; arachidonic, linolenic, linoleic, oleic), peptides (systemin, elicitin), salicylates, jasmonates, ethylene and even electrical signals are produced and act either directly or as signalling mediators for SAR and ISR.

Probably the most common signalling molecule for SAR is salicylic acid (SA), biosynthesized from the amino acid phenylalanine. SA is today recognized as a phytohormone, besides its well known antipyretic and analgesic effects in animals (Raskin, 1992). However, its most important roles in plant organisms seem to be as both an endogenous signal and an external elicitor of SAR and ISR through the induction of PR genes (PRs stands ffor "pathogenesis related proteins"). Although these processes seem limited to temperatures under 32ºC, Quarles (1996) reports that either aspirin or willow bark extracts (*Salix alba*, *S. vitellina*), not only promote resistance in garden plants but also attract useful predator mites for biological control.. However, there are different ISR and SAR elicitation pathways, independent from SA accumulation (Rice, 1984 & 1995; Sticher *et al.*, 1997).

4. Abiotic and Biological Elicitors

Elemental abiotic elicitores like *Si, Cu, Ag, Hg* (Rouxel *et al.,* 1989 & 1991) and even and synthetic agents like polyacrylic acid also follow SA independent ISR pathways, as their effects still take place at temperatures above 32ºC (Sticher et al., 1997).

Sulphur is an essential part of many active volatile and allelopathic compounds like (Hengel & Kirkby, 1982):

1. Mustard oils, glucosides or glucosinolates, present mainly in members of the *Cruciferae*; *e.g.*; sinigrin (in *Brassica nigra*), gluconasturtin (in *Nasturtium officinale*), glucobarbarin (in *Reseda luteola*), glucosinalbin (in *Sinapis alba*), glucotropaeolin (in *Tropaeolum majus*, *Tropaeolaceae*).

2. Sulphoxides, *e.g.*; the lachrymatory factor in onion and the odour of garlic, which contains allicin - both a molluscicide and an insecticide (Singh *et al*., 1995).

3. Elicitins, which are small hydrophilic cystein-rich proteins.

4. Also for the production of ethylene from the amino acid methionine.

An example of sulphur's importance in plant resistance processes is the effectiveness of metabolized elemental S, identified in resistant genotypes of cocoa (*Theobroma cacao* L.) to verticillium wilt (*Verticillium dahliae* Kleb.; Resende *et al*., 1996).

Plant promoting rhizobacteria (PGPR) and vesicular-arbuscular mycorrhiza (VA) present in soil organic matter and plant communities also effectively promote ISR and are most probably the cause of observed crop protection using diversification and induced resistance in low-input cereal/legume cropping systems (Cooke, 1996; Sticher *et al*., 1997).

Last but not least, the importance of Silicon (*Si*) in the plant systemic resistance context is being rediscovered (Epstein, 1994) – given that Silicon was already mentioned by Liebig (1842) in the very beginnings of Agricultural Chemistry and recommended in biodynamic treatments by Steiner (2000) in 1924; not only as an efficient resistance elicitor, but also as a general metabolic regulator and essential nutrient element for both plants and animals (Simpson & Volcani, 1981; Salisbury & Ross, 1992; El Behairy, 1994).

5. Allelodynamics: the A,B,C of Biodynamics.

As the processes which elicit the biochemical signal cascades involved in ISR e SAR neither take into account nor are directed to the plant's cosmic connections presupposed and aimed at by the Biological-Dynamic agricultural system, it was suggested that the observed effects in the activity of ultra-high dilutions in biological systems (Endler & Schulte, 1994) be defined as <u>allelodynamic, *i.e.*, dynamic effects of highly diluted allelopathic substances</u> (Deffune, 2000a).

Thus, Biological-Dynamic Agriculture - with its original concept of healthy internal relationships in "agricultural organisms or individualities" (Scofield, 1986) and systematic application of apneumonic extracts on crops, was also the modern precursor of Applied Allelopathy - considering that the management of general allelopathic interactions was already known in times as early as Theophrastus' (*ca.* 374-287 BC), Aristotle's favourite disciple and successor (Keynes, 1929; Rice, 1984; Deffune, 1990 & 2000).

Therefore, it is interesting to note that the old criticism and discredit by the agrochemical establishment toward concepts of "natural control" for pests and diseases based on the stimulation of the plants' natural resistance in the Organic and Biodynamic systems, are largely due to a lack of information on the recent advances in the fields of ISR and SAR (Pio *et al*.,1984; Deffune *et al*.,1994; 1996; Langer, 1995; Cooke, 1996; Sticher *et al*., 1997).

On the other hand, the supposed efficacy (which has already been submitted to research without positive confirmation) of the so-called "natural inputs" - somewhat complex organo-chemical recipes and microbial inoculi (*e.g*.; "Agroplus ®", "Super-8", "Super-Magro", "EM") recently popularised in the organic agricultural circles, may well be due to simple effects of either abiotic (*e.g*.; the sulphur and copper present in most of these recipes) or biological elicitors (organic metabolites and/or saprophytic microbes). These effects can in principle be obtained through simpler and cheaper management strategies and preparations, like Silica suspensions (ground quartz or Diatomaceous earth), mature Compost extracts, or 0,1% diluted lime-sulphur and Bordeaux mixtures. Similarly, part of the experimentally confirmed effects of Biodynamic Preparations, are also due to elicitation processes and signal cascades at the genetic, hormonal and biochemical levels (Raupp & König, 1996; Deffune 1981, 1990, 1999 e 2000).

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POSSIBILITIES OF THE USE OF PHEROMONES FOR PEST CONTROL IN BRAZIL

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What are pheromones?

Pheromones are chemical substances that are used in the animal world to transmit information from one individual to another. Mainly insects, first of all moths but also beetles are using this transmission to communicate. Amongst the different types of pheromones, like aggregation, alarm and tracing pheromones, the sex pheromones play the most important role in the field of plant protection. Sex pheromones are released by female insects signalling their readiness for copulation and allowing the males to track them down.

The pheromones, which are carried by the motion of air over great distances, are detected by the antennae of the males. These antennae are very sensitive instruments and in some cases able to recognize even single molecules.

What are they good for?

Pheromones can be synthesized synthetically and are used in pheromone traps as bait. The most important part of the trap is the so called dispenser which contains a small amount of the attractant. It mimics a female insect, misleading and attracting the males to be caught in the trap. Pheromones are basically species specific and the males are attracted by the substances that are released by their own species only. Therefor traps can be built, attracting the target moth or beetle only.

Monitoring

The most common use of pheromone traps. A few traps are placed in the area of interest. By counting the number of insects caught over a certain period, information about the presence of a insect pest, its flight activity and the pest population density is easily available (flight curve). Based on this information appropriate measures can be taken.

Certified Dispensers ensure, that the results obtained during one season can be compared with those of the next year. They are produced from the same batch of pheromone and are continuously tested in the laboratory and in the field.

Advantage

Information about the flight activity of a particular pest is easily available. No time consuming examination of the plants is necessary and no expert knowledge is needed. Appropriate proceeding is possible according to the outcome of the count.

No catches of the target pest are observed: It is most likely that no damage from this pest has to be expected. As a consequence no pesticide treatment is necessary. This saves work and money and keeps your plantation free of nature burdening pesticides.

Target insects are caught in the traps: This means some damage has to be considered caused by the larvae hatching from the eggs. In consequence control measures are necessary. The flight curves indicate the optimum time period for pesticide application. Spraying throughout the whole season can be avoided.

Mass trapping

For several pests like bark beetles or palm weevils the usage of a greater number of traps has proven to be effective. Especially in areas where the target insect population is low the number of insects can be reduced markedly. Thus mass trapping can be sufficient to reduce the damage, they or their breed respectively may cause, to an acceptable level.

Advantage: Plant protection without the need of a pesticide and without polluting the environment. Due to the specificity of pheromones no other harmless or beneficial insects are killed.

Attract & kill

All methods mentioned above are using any kind of trap to keep the once attracted insects inside. Attract & kill works different. Using pheromones the target insect is lured to a place where it gets into contact with a poison (widely used is Permethrine). Males contacting poisoned area die within hours. Thus, reproduction is reduced.

Mating disruption

An additional and in its effect different way of using pheromones for plant protection. Many dispensers releasing a large amount of pheromone are placed all over the area that has to be protected (150 to 1000 dispensers per hectare). A high and permanent concentration of pheromone is generated which prevents the meeting between individuals of the opposite sex by masking the natural pheromone. Insects are not able to locate their females which remain not impregnated and cannot reproduce themselves.

Using this technique vineyards and orchards are protected successfully from damage caused by grape berry moth, grapevine moth and codling moth without using any pesticide. Mating disruption of Pink Bollworm in cotton is successfully used in Egypt. The method is used and tested against a variety of other insects.

POTENTIAL OF USE, PRODUCTION OF EXTRACTS AND DEVELOPMENT OF PRODUCTS OF NEEM BASED MEDICAMENTS AND USES IN AGRICULTURE

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In the medicinal chemistry, original products of plants increase in the therapeutics, creating a growing trend for in research of new pharmaceuticals and active substances with medicinal properties.

In this context the plant Nim *Azadirachta indica* A. Juss was introduced in Brazil in 1986 in the Agronomic Institute of Paraná (IAPAR), it has been waking up the interest of Researchers in Brazil, in studying its use as natural insecticide for the control of ectoparasites.

Nim is a plant of Asian origin belonging to the family of the meliáceas, natural of Burma and of the arid areas of the Indian sub-continent, being considered a plant with important insecticidal properties. It is very resistant and of fast growth and reaches usually 10 to 15 m of height and depending on the soil type can reach up to 25 meters in height. Its red, lasting and resistant wood is a succedaneum of the mogno, with the advantage of faster growth.

Of the tree Neem, or " tree of the life " as it is known in India all parts are used. Seeds, fruits, bark and roots are used as raw material in the production of insecticides, fungicides, carrapaticides and fertilizers in the combat of dangerous plagues. Medicinal products are also produced, such as vermifuge, healing, antibacterial and further on products for personal hygiene, as soap, shampoo, dental cream.

For the use as a botanical insecticides several aspects should be taken into consideration: extraction, conservation of the extracts, efficient dose, stability, toxicity, cost. All these aspects are understood when the main substances contained in this insecticide is identified.

Recently a work was developed in the University of Uberaba, through the comparative study of the physical-chemical properties of the oil of the seeds of Nim of different areas. Three samples of oil of the seeds of Nim were studied comparatively.

Those oil samples were obtained through an exhaustive extraction in an extractor of the type soxhlet using n-hexane as extraction solvent. After the extraction of the oil of the seeds, the solvent was evaporated and they were used in comparative studies with samples of oil of different areas. The Nim oil samples came from the Dominican Republic, another from Fortaleza (Ceará-Brazil) and another from the National Center of Researches of Rice and Bean - EMBRAPA (Goiás-Brazil).

Some comparative analyses of these oils were accomplished, such as determination of the peroxide index, determination of the iodine index and of the saponification index. That study of the physical-chemical analyses of the oil of the seeds of the nim cultivated in different areas is fundamental to demonstrate some differences which may exist in the chemical composition of the oils of these seeds.

Through the analysis of the results, it was possible to observe that the samples of the oil of the Nim seeds of different places presented the same results basically in relation to the peroxides indexes and of iodine. In relation to the peroxide index, it was observed that the oils of the nim seeds present a very small index, demonstrating an additional stability when compared to a sample of sunflower, *Heliantus annus* oil in similar conditions.

However, for the saponification index for the three samples, was observed that the oil sample originating from the Dominican Republic presented an index of saponification superior to the anothers. In that way, some differences in the constitution of the oils, of quantitative or qualitative greatness were observed. That allows to say, that the composition of the active substances of the plant depends on the place of cultivation of the same, possessing different concentrations of the active ingredients between one place and another.