Practice Oriented Results on Use and Production of Neem-Ingredients

H. Kleeberg (Ed.)
Trifolio-M GmbH

Proceedings of the 1st Workshop
Wetzlar, June 19th - 20th 1992

Druck & Graphic, Giessen
1992

Practice Oriented Results on Use and Production of Neem-Ingredients

Proceedings of the 1st Workshop; Wetzlar, Germany, June 19th - 20th 1992

Printed by:
Druck & Graphic, 6300 Giessen, Kiesweg 22
1992

Compiled and prepared by:
Hubertus Kleeberg

Distribution:
Trifolio-M GmbH, Sonnenstr. 22, D-6335 Lahnau 2

ISBN: 3-925614-09-5

Printed in Germany

CONTENTS

List of Participants and Contributors:

Achimu, Pelina
Wiss. Zentr. Tropeninst. d JLU, Phytopathol. u. Angew.Ent., Ludwigstr. 23
6300 Giessen , *BRD*
Tel.: +49-641-22719;

Bode, E.
Biol. Bundesanst. f. Land- und Forstwirtschaft, Messeweg 11/12,
3300 Braunschweig , *BRD*
Tel.: +49-531-3991; Fax.: +49-531-399239

Chianella, Massimo
Siapa- Research Centre , Via Vittorio Veneto 7,
40015 S.Vincenzo di Galli , *ITALIA*
Tel.: +39-51-812696; Fax.: +39-51-815508

Eisenlohr, Ute
GRAB, Agroparc , B.P. 131 84146,
Montfavet , *FRANCE*
Tel.:33-90236721; Fax.:33-90840037

Ermel, Klaus
Inst. f. Phytopathologie. und Angew. Zoologie, JLU, Ludwigstr. 23,
6300 Giessen , *BRD*

Grunewald, Jörg
Institute of Tropical Med. , Wilhelmstr.27,
7400 Tübingen , *BRD*
Tel.: +49-7071-294949; Fax.: +49-7071-293017

Hummel, Edmund
Trifolio-M GmbH , Sonnenstraße 22,
6335 Lahnau 2 , *BRD*
Tel.: +49-6441-63114; Fax.: +49-6441-64650

Hummel, H.E.
Inst. f. Phytopathol. u. Angew. Zoologie
Biol. u. Biotechn. Pflanzenschutz der
Justus-Liebig-Universität
Ludwigstr.23, 6300 Giessen, *BRD*
Tel.: +49-641-702-5977

Jakob, Gerhard
BBA, Inst.f.Pfl.schutz im Obstbau, Schwabenheimer Str.101,
6915 Dossenheim , *BRD*
Tel.: +49-6221-85238; Fax.: +49-6221-861222

Kienzle, J.
LVWO Weinsberg , Traubenplatz 5,
7102 Weinsberg , *BRD*
Tel.: +49-7134-8935; Fax.: +49-7134-50433

Kleeberg, Hubertus
Trifolio-M GmbH , Sonnenstraße 22,
6335 Lahnau 2 , *BRD*
Tel.: + 49-6441-63114; Fax.: + 49-6441-64650

Kovalev, Boris
Inst. of Biological Methods in Plant Protection, Prospect Mira 58,
277072 Kishinev , *MOLDOVA*
Tel.: + 7-0422-570-433; Fax.: + 7-0422-570-433

Lang, Bernd
Heilig Geist Str.5,
6900 Heidelberg , *BRD*
Tel.: + 49-6221-12202; Fax.: + 49-6221-161619

Leupolz, W.
Schäfereigenossenschaft Finkhof e.G., St. Ulrich Str. 1,
7954 Arnach/Bad Wurzach , *BRD*
Tel.: + 49-7564-4530; Fax.: + 49-7564-4967

Lingren, Bill
Trece Inc. , 6278, 1143 Madison Lane
Salinas CA 93912, *U.S.A.*
Tel.: + 1-408-758-0204; Fax.: + 1-408-758-2625

Mandellaub, Bettina
c/o Wolf Möhn , Herrleinstraße 43,
8750 Aschaffenburg , *BRD*
Tel.: + 49-6021-26649;

Mathur, Vishnu
CBC The Nature of Things , 500, Station "A",
Toronto MSW IE6, *KANADA*
Tel.: + 1-416-975-6893; Fax.: + 1-416-975-6887

Mebrahtu, Yohannes
Heilig Geist Str.5,
6900 Heidelberg , *BRD*
Tel.: + 49-6221-167273; Fax.: + 49-6221-161619

Ostermann, Heike
Schulstr. 11,
5431 Welschneudorf , *BRD*
Tel.: + 49-2608-524;

Schröder, Peter
IFAH , Großherzog-Friedrich-, Straße 2A
7750 Konstanz , *BRD*
Tel.: + 49-7531-44439; Fax.: + 49-7531-44890

Serra, Colmar A.
Ippendorfer Allee 36,
5300 Bonn 1 , *BRD*
Tel.: + 49-228-285135;

Teutsch, H.
Bezirkspflanzenschutzamt Koblenz, Rathausstraße 1,
5401 Emmelshausen , *BRD*
Tel.: + 49-6747-6505;

Wendorf, Martina
Fachgebiet Methoden des Alternativen Landbaus, GHK, Nordbahnhofstr. 1a
3430 Witzenhausen , *BRD*
Tel.: + 49-5542-503 570;

Wilps, H.
Gresserstr. 13,
7800 , *BRD*
Tel.: + 49-761-39607; Fax.: + 49-761-203-2496

Welcoming Address

Ladies and Gentleman, the summit of Rio is over, but the world has not changed! During the recent days we all have heard the very well prepared contributions and the excellent discussions of the summit in Rio. Documents were signed in order to start an ecologically sound world economy which is considerate with respect to raw-material.

One may hope that it became clear to more people that we are all living in the one world and we are responsible for it - more or less in the same proportion in which we use nature.

Now, what is the reason for this "1st workshop on Practice Oriented Results on Use and Production of Neem-Ingredients"? Did politicians not say all? I hope that the discussions in Rio have been useful, but they are surely not sufficient. We have to act - with or without the support of politics! It became clear, that much has to be done. We have to learn more about the threats to man and nature since ignorance is the largest threat.

One major point of discussion in Rio centred on forests. It is clear that plants are of utmost importance for the welfare of man and nature: as a by-product to the purification of our atmosphere they supply us with nutritious food and fodder, fuel wood, timber, paper and cloth as well as medicines and pesticides.

Not only for this reason we have to take care of trees and plants, we have to protect them. There are some doubts that our "traditional" (chemical) possibilities of protection of plants are the ultimo ratio. Plants themselves offer to us a large variety of possibilities for their own protection. During the last decades it has convincingly been worked out by engaged scientists that NEEM may be one possibility to bring together afforestation and plant protection, health and food and - this seems to be of special importance - learning from each other: northern hemisphere from southern hemisphere. In India, in many countries of Africa Neem is used since generations, since thousands of years.

We have met here to discuss a topic which is well known in principle. Why?

I think not only someone who has a Neem tree aside his house should benefit from it - let's see whether we can make more of it: for the sake of man and nature!

Not only that it is true since times immemorial that Neem has very versatile possibilities; very recently these possibilities have been described by the "National Research Council" of the USA. Now, you find articles about Neem in nearly every newspaper.

But what about the practice? We still have to learn much - even about the well investigated insecticidal properties of Neem. One problem is that we do not have sufficient Neem trees; we have to be very cautious with this renewable source of pesticides. We should try to use as little quantities as possible to obtain sufficient protection of our crops. We have to try to store Neem-ingredients as carefully and as long as possible - which is not as simple as it seems. Of course we have to process the Neem-seeds in an environmentally friendly manner. And we should not forget, that the basic knowledge about Neem comes from so called "developing countries".

The idea of this workshop is to try to adabt the ancient knowledge to nowadays needs and conditions. It is the aim of this meeting to exchange results and ideas on application and efficiency, production and registration obtained with ingredients of the Neem-tree and possibly co-ordinate future activities.

I wish you very fruitful discussions during your stay in Wetzlar and hope that you return home with new ideas about what has to be done.

Wetzlar, June 19th 1992 Huberetus Kleeberg

NEEM-ACTIVITIES WORLD-WIDE

C.A. SERRA

Institut für Phytopathologie und Angewandte Zoologie
Justus-Liebig-Universität Giessen, Germany

ABSTRACT
Since 1987 the Fed. Rep. of Germany supports the project "Natural pesticides from tropical plants". The objective of the project is to introduce the use of seed extracts and seed oil from the neem tree (*Azadirachta indica* A. Juss.- Meliaceae) as an insecticide for pest control to be used by farmers of pilot regions. To reach this aim the supply of farmers with neem seeds as raw material should be improved, methods for the production and application of simple neem products developed, as well as investigations concerning the socio-economic acceptance implemented. In addition, it is intended to spread the knowledge about the appropiate use of neem among farmers and institutions which are interested as well as promoting neem programs of non-governmental organisations (NGOs) and projects.
The realisation of the project is coordinated by the Institute for Phytopathology and Applied Zoology from the University of Giessen. The project has a station operating in the Dominican Republic at its disposal and it is here where the main activities are executed. Apart from that, the project runs limited activities in several developing countries in Africa, Latin America and Asia.
Because of its pilot character the project has been planned as a long-termed sectoral project to ensure an efficient introduction of the use of neem insecticides among farmers.
In the period from Aug./90 to July/93 the financing of a coordinator, laboratory staff, 2 long-term experts and short-term experts, the supply of material for farm and laboratory work, the training of counterparts and material required for giving advice are planned.

AIM AND REASONS FOR STARTING THE PROJECT

In spite of the suitability of natural insecticides for pest control the farmers of developing countries make only a rather limited use of them at present. The reasons for that have to be seen on one hand in the lack of marketable products that could compete with synthetic insecticides. On the other, the manual process for the extraction of botanical insecticides is considered to be old fashioned and intensive in work. Favourable conditions for the use of natural insecticides are given in regions where pests have become resistant to synthetically produced active ingredients and where various toxicological problems for human beings and the environment have arisen due to the excessive application of commercial insecticides. The lack of neem trees and consecuently of raw material has to be regarded as a handicap for the use of highly efficient neem products in several countries. By the end of the project the following aim should be reached: "farmers of the pilot regions generally use neem insecticides as a control method against diverse sorts of pests". Indicators to verify the attainment of

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

the goal for the project are:
The demand of neem insecticides in the Dominican Republic will reach during the last year the equivalent of 100 t of raw material and in countries with cooperating NGOs 12 t world-wide. Target groups of the activities are farmers with little access to synthetic insecticides or those having problems connected with the use of synthetic products as well as specialists in plant protection and agricultural advisers from the official or the private sector. Apart from that, co-operatives and artisanal neem-processing plants belong to the target group as well.
The project corresponds to fundamental aims and principles of German technical co-operation. By cultivating and using neem trees systematically the project supports the conservation of resources. The application of neem insecticides helps to protect yields and contributes to protect the environment.

REALISATION OF THE PROJECT
At the beginning of the project primarily analytic works and extended investigations on the effects of botanical compounds were carried out. Several insecticidal compounds from neem fruits were identified. Later on the main activities of the project consisted in developing practicable methods for the application of neem insecticides and the attempt to introduce them in practice.
Nowadays the project is based on an extended program to spread neem insecticides. The target groups should be able to produce simple neem products by themselves and use them in pest control.

Aspired results (1994):
- The supply of the farmers in the project regions with neem seeds has improved.
- Methods for the production and application of simple neem products are developed.
- The knowledge on the potential use of neem insecticides is increased.
- The farmers of the pilot regions have sufficient informations about the appropiate use of neem at their disposal.
- The socio-economic acceptance of neem insecticides is implemented.
- The obtained knowledge on the use of neem has been passed on to interested private and official institutions.
- There has been a support for independent neem programs of NGOs and other projects.

The maintenance of partner institutions in developing countries is contemplated following the end of the present project phase. For that purpose the NGO-programs will be continued through own projects, measures of the GTZ or with the support of other donors.

RESPONSIBILITY OF THE PROJECT
The project is managed by the Institute for Phytopathology and Applied Zoology from the University of Giessen where the GTZ-coordinator and the administration are located. The station of the project in the Dominican Republic is affiliated to the Instituto Politécnico Loyola (IPL). Within the agricultural

department the IPL has the section "Investigation and diffusion of natural insecticides" at its disposal with several techniciens. Other associated organisations are NGOs, among them the Centro Manabita de Desarrollo Comunitario (CEMADEC) in Ecuador, the organisation Gami Seva Sevana (GSS) in Sri Lanka, "Natural plants" in Thailand and Caritas in Niger.

EFFECTS OF THE PROJECT, ACCEPTANCE AND RISKS

The competitive pressure of pesticide distributors plays an outstanding role and has to be given special consideration. On the other hand also in developing countries an ecological consciousness is rising. Activities of ecological oriented groups and institutions might influence the work of the project positively.

A broad acceptance of neem insecticides is only attainable if the products are economically competitive. In this context key factors as the trend of the prices, as well as possible prohibitions and subsidies for synthetic insecticides have to be taken into account and steadily actualized for the respective countries.

The probability of reaching the aim of the project has to be considered as good due to the increased sensibility concerning the use of pesticides. Negative consequences for the target group are not likely to occur because of favourable toxicological properties of neem for human beings and the environment.

Farmers who do not use insecticides or those who have problems with resistance in their fields could prevent yield losses through the utilization of neem. A self-sufficient supply of insecticides permits farmers to stabilize their yields without depending on the insecticide market. The harvest and processing of the neem seeds which are supported by the project, will possibly be executed in some countries by women.

The positive environmental effects are expected due to the substitution of poisonous synthetic pesticides which are often harmful for the environment. According to the actual knowledge and experiences the ecological risks of the cultivation and utilization of neem trees have to be considered as very small.

PRESENT STANDING OF PROJECT

During the actual phase of the project more than 21 t of neem seeds have been harvested or bought and placed at cost at farmers' disposal for them to produce insecticides. At the same time about 220 000 neem trees were distributed for planting. Simple methods for the production of neem insecticides were developed and in a large measure adapted to the situation of small farmers. A fundamental concept for an artisanal small-industrial production of neem insecticides as a semi-finished or finished product exists although there still are problems concerning the standardisation and stabilisation of the latter. Numerous field trials in the Dominican Republic, Ecuador and Sri Lanka helped to obtain an important knowledge about the potential utilization and dosage of several neem products leading to recommendations for more than 10 field crops and 20 pest species.

Farmers in the project regions were intensively adviced about

the production and utilization of neem through seminars, field-days, demonstration-plots, booklets etc. More than 61 pilot-farmers in the pilot-regions were given support and advice by the project. More than 1000 farmers have participated in a training program concerning the practical production and utilization of neem insecticides.
The project has also trained 12 associated techniciens (generally for 3 month), answered to over 500 inquiries. There exist informal contacts to 22 NGOs with neem-programs. The project supports mainly neem-programs of 5 NGOs based on co-operative contracts in Ecuador (CEMADEC), Sri Lanka (Gami Seva Sevana), Nepal (INSAN), Thailand (Natural Plants) and Haiti (MPP). The cooperation with the MPP had to be interupted due to the military revolte in Haiti. The project maintains a close contact to 4 GTZ-projects with neem-components in Myanmar (Burma), Madagascar, Benin and Nepal.
Laboratory staff at the University of Giessen has been in charge of analysing the amounts of the most important active neem compound (azadirachtin) in seed samples from many localities and countries or even from single trees by using "high performance liquid chromatography" (HPLC). In addition, finished or semi-finished neem-products have been produced and studies to improve their extraction, stability, formulation, effectiveness etc. have been implemented. The AZA-content of commercial neem concentrates from India, the USA and other countries have been analysed as well.

REFERENCES

GTZ (German technical cooperation, 1992): Projektfortschrittsbericht, unpublished report about the progress of the project "Natural Insecticides from Tropical Plants" of the GTZ.

The NeemAzal Conception: Test of Systemic Activity

Hubertus Kleeberg
Trifolio-M GmbH
Sonnenstr. 22
D-6335 Lahnau 2

Summary

Insecticidally active ingredients from the Neem tree (*Azadirachta indica* A. Juss) fulfil even the most severe requirements for modern pesticides. In addition to the inexpensive, toxicologically and environmentally safe production of the highly active insecticide NeemAzal in standard quality, the use of the Neem tree as a renewable source has the following advantages: afforestation of semi-arid zones, supply of high quality timber or of fire-wood, inexpensive production of Neem-oil as a starting material for industrial products (like soap) and Neem-cake as an excellent fertilizer with nematicidal and denitrification inhibitory properties.

The production of NeemAzal in "Neem-countries" may be considered a crystallization point for further improvement of the infra-structure. NeemAzal-products may be used for plant protection in Neem-countries or may be exported. NeemAzal-F fulfils even the highest claims for modern pest control measures. As indicated by first results the systemic application of NeemAzal seems to be very efficient and may be preferable in special cases over the usual application of spraying NeemAzal-F after dilution with water.

Introduction

There is an increasing need for means for ecologically sound plant protection. The developmental cost for chemical insecticides increases drastically from year to year (Gubler K., 1983). This makes the development of specific pesticides difficult on behalf of economic reasons. However, in order to increase the yield and quality of agricultural products new principles are badly needed (Horn D., 1988).

In this situation it seems useful to study natures own possibilities of pest control either to determine efficient lead structures or to extract efficient active ingredients from natural sources. We have chosen to concentrate on the second possibility since

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

extractions can usually be carried out with much lower amounts of waste than chemical reactions. Usually all by-products of extractions from living matter can be used in one way or the other and thus do not create problems in the environment.

In order to become a valuable tool in future pest management a new pesticide should fulfil the following ten requirements:

1.) Efficiency: Very clearly pesticides have to be efficient! Without efficiency it may be possible to market a product profitably, but it is not possible to control a pest.

2.) Reliance on the success of the application: Under the specified conditions under which a certain pesticide should be used the result of the application should be predictable with a high degree of confidence.

3.) Considerate influence on benefitial insects: Since benefitial insects can control the growth of a pest under favourable conditions, a pesticide should not have a persistent negative influence on them.

4.) Minimization of the appearance of resistancies: It should be an intrinsic property of a new pest control measure that the development of resistancies is highly impropable.

5.) Toxicological safety for the user: Agents of low toxicity towards man will prevent poisoning of workers during production, packing and especially application.

6.) Toxicological safety for the consumer: Residues of the pesticide should not be harmful to man or animal.

7.) Environmentally sound (biodegradability, no accumulation): The safest way of decomposition of complex substances usually is biodegradation, i.e. the compound is metabolized by microorganisms, plants or animals and inserted into the natural food chain.

8.) Toxicologically and environmentally safe production: For the safety of man and nature the production should be as safe as possible; usually production processes are the easier to controll the simpler they are.

9.) Inexpensive production: In order to make a pesticide practically available especially in those parts of the world which need it most badly, it is a necessary prerequisite that it is inexpensive.

10.) World-wide availability of standard quality: The above points can only be fulfiled in general, if the quality supplied is reliably the same at any place and time.

The first 7 requirements are usually taken into account in the course of authorization of a pesticide in most countries. The importance of each point has to be discussed critically for each intended application.

The last 3 requirements are usually not taken into account during official evaluation of a pesticide. However, they are important in view of the solution of global problems.

According to the large body of information on properties of pesticides from the Neem tree (*Azadirachta indica* A. Juss) (Schmutterer H., Ascher K.R.S. and Rembold H., 1980; Schmutterer H. and Ascher K.R.S., 1984, 1988; Jacobson M., 1989; Menn J.J., 1983; National Research Council, 1992) it seems promising to develop technical methods for the extraction of the biologically active principle from the seeds of this tree. From the literature this is true especially with respect to the first seven requirements mentioned above.

The major **insecticidal ingredients** of Neem are termed Azadirachtins (see: Broughton et al., 1986; Jones et al., 1989). According to present knowledge this group of compounds and similar ones usually extracted together are non-toxic to mammals and are readily degraded on plants and in the soil (Kleeberg, 1992a). Due to the high efficiency of these substances (i.e. low amounts applied to plants for their protection against insect pests) and the fast bio-degradability the requirements with respect to toxicological and environmental safety are fulfiled. Recent studies indicate that the effect of these Neem ingredients on benefitial insects are negligible (Schmutterer H., personal communication). Laboratory tests and theoretical considerations indicate that resistancies are not to be expected. Many examples for the efficiency and reliance on the success of the application of NeemAzal-F are described or cited in this volume.

Neem grows in a limited belt around the equator, in countries which frequently need means for pest control very badly. Thus it is politically compelling to produce Neem-insecticide inside these countries. It will not change the unbalanced situation between north and south if the raw material is exported from these Neem-countries and processed in industrialized countries. Since Neem may be used advantageously for afforestation more area may be cultivated in Neem-countries by Neem-plantations in the form of agro-forestry or intercropping. Thus with Neem, this renewable source of pesticides, we may contribute to the solution of many more problems than only those of plant protection.

The NeemAzal-Products:

Under these conditions it was our task a few years ago to solve requirements 8 to 10 of the above list. As illustrated in figure 1 we have developed two alternative ways for the extraction of the insecticidally active Neem-ingredients.

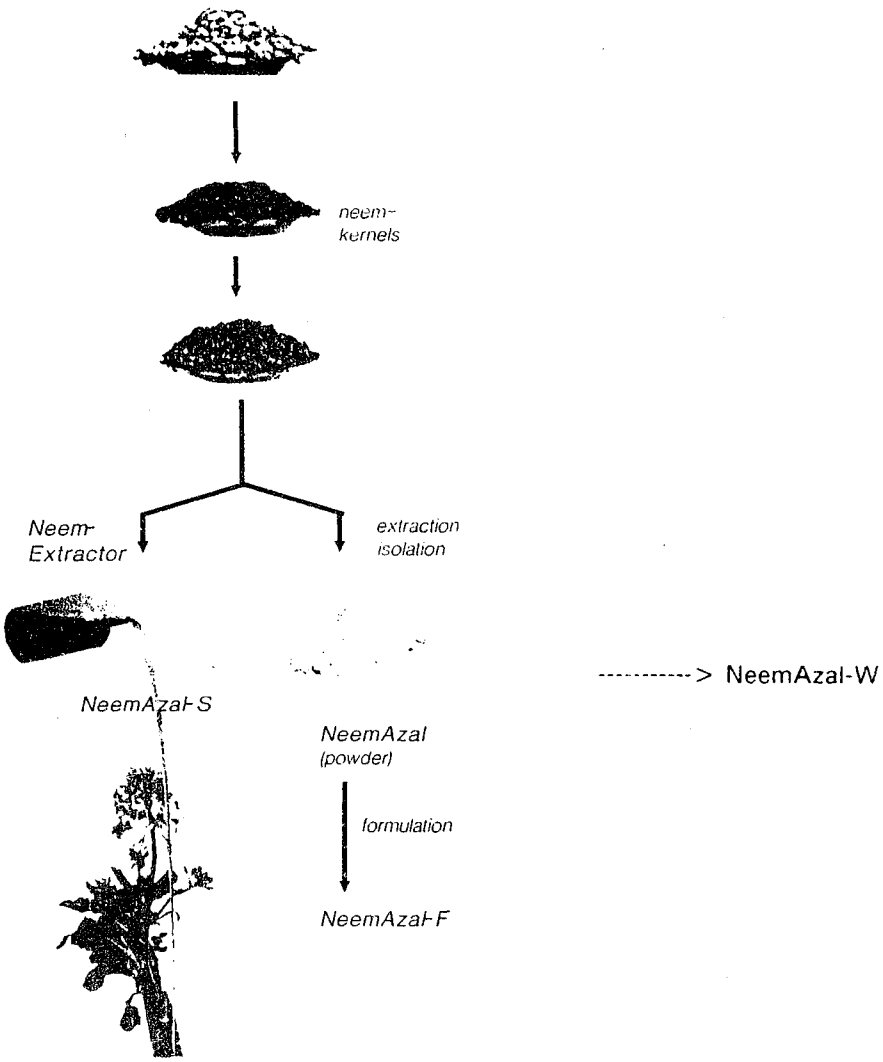


Figure 1: Inexpensive ways from the raw material "Neem-seeds" to the ready for sale botanical insecticide.

NeemAzal-S:
This is a ready to use solution which may be applied after dilution with water. The content of active ingredient depends on its concentration in the Neem-seeds used. The simple production is made possible by the Neem-Extractor which may be used on the village or cooperative level. In order to keep first investments low a reasonable turn-over of the Neem-Extractor will be of the order of 20 kg per day - which will suffice to treat about 3 hectar of crop. Under these conditions the NeemAzal-S produced will be economically viable.

A storage stability of NeemAzal-S of about 10 month seems reasonable for marketing or distribution in the area of the production site. In case analytical facilities are available it is easily possible to standardize the content of active ingredient at about 3000 ppm Azadirachtin. However, the provision of analytical equipment for one

single Neem-Extractor will probably not be economic. The efficiency of NeemAzal-S is comparable to the first Neem-product marketed in USA (Dimetry N.Z. and Schmidt G.H., 1992, Karelina T.N. et al., 1992; Kleeberg H., 1992b). The Azadirachtin content and storage-stability of NeemAzal-S is usually better than that of products currently available in India and Thailand, for example (Kleeberg H., 1992c).

NeemAzal:
NeemAzal is a powdery concentrate of the insecticidally active ingredients of Neem containing between 25 to 60% Azadirachtin. The Azadirachtin content depends on details of the production process, which had been designed for intermediate to large scale production of turnovers of the order of one ton Neem-seed kernels per day. Practically standardization of the Azadirachtin content of NeemAzal to 30% seems to be a technically and economically reasonable compromise. The storage stability of NeemAzal-F at room temperature is about 2 years and may be increased by cooling. NeemAzal-powder is the active ingredient for the following formulations:

NeemAzal-W:
NeemAzal-W is a water soluble powdery formulation which is currently under development especially for application using the systemic effect of Azadirachtin (see below and Wendorf M. and Schüler C., this volume). An aqueous solution of NeemAzal-W (about 0.03%) may be used directly for drip irrigation or watering of plants.

NeemAzal-F:
NeemAzal-F is a liquid, water soluble formulation of NeemAzal containing 5% Azadirachtin. Its storage stability is 2 to 3 years under room conditions. Many field and laboratory tests show that sufficient efficiency of NeemAzal-F is obtained by spraying as a 0.1 to 0.3% aqueous solution. For example against thrips application of 0.05 to 0.15% NeemAzal-F has proven very efficient (Böhmer B., et al., 1991).

The products described will be produced safely and to reasonable costs. Especially in the case of NeemAzal-F (and very probably NeemAzal-W) world-wide availability in standard quality will not be a problem.

As the only by-products of the production of NeemAzal Neem-oil and Neem-cake is obtained which may readily be used in agriculture and industry (Ketkar C.M. and Ketkar M.S., 1989).

Properties of NeemAzal:

NeemAzal consists of about 50 chemically different substances which vary in concentration between 0.1% to about 40%. However, in different batches of NeemAzal, which have for example been prepared from Neem seeds of very different quality (between 2 and 8 g Azadirachtin per kg Neem seed kernels) or origin (Asia,

Africa or Latin America), the content of each compound of NeemAzal does not vary much, i.e. the ratio between the different compounds present is constant within reasonable limits. In all cases the Azadirachtins amount to about 50%.

Several of the chemical entities of NeemAzal have been isolated, characterized chemically and tested biologically in the laboratory. Some compounds are as efficient or even slightly more efficient in tests with Epilachna varivestis as Azadirachtin. Surprisingly enough the bio-efficiency of NeemAzal is comparable to that of pure Azadirachtin. This may indicate that synergistic effects may play a role between different compounds.

In aqueous solution, in soil and on the plant the half life of Azadirachtin is approximately 10 days (Kleeberg H., 1992a). This reasonably fast degradation seems to be very close to the optimum of the compromise between ready degradability - which is especially advantageous with respect to toxicological safety of the consumer and a low load of the soil - and sufficient persistence with respect to lowering the population of the pest.

As a rule of the thumb an as early as possible application of NeemAzal-F seems desirable since the small larval stages react much more sensitve on the Neem ingredients than the larger ones. In a lag phase of 4 to 8 days between the intake of the active compounds and the final disappearance of the pest the population of the pest may additionally serve as a pray for the increasing population of benefitial insects.

The time dependence was investigated on small trees of Salix caprea which were infested by Tenthredinidae (probably: *Croesus septentrionalis).* The infested twigs were sprayed with NeemAzal-S corresponding to 2 ppm Azadirachtin in the spraying solution. Blank: spraying with an aqueous solution of the same composition as NeemAzal-S except for the ingredients of Neem seed kernels. The larvae had hatched two days before treatment. The average temperature was about 8°C at night and 18°C during the day (Trifolio-M, 1989). The results are summarized in Figure 2.

In the upper part of figure 2 the time dependence after treatment of the average weight of the larvae is indicated. It is obvious from figure 2 that the treated larvae stopped growing two days after treatment, while the body weight of the untreated control increases constantly. The total leaf mass consumed after treatment was only 1.3 g per g of larvae while the untreated larvae had consumed 2.5 and 4.3 g of leaf mass after 3 and 10 days, respectively. After 25 days the blank larvae had digested more than 7 times their actual body weight (about 50 mg/larva) of leaf mass. During this time "blank larvae" started to pupate. Correspondingly the over all damage after NeemAzal-S treatment was only 5% of that of the blank.

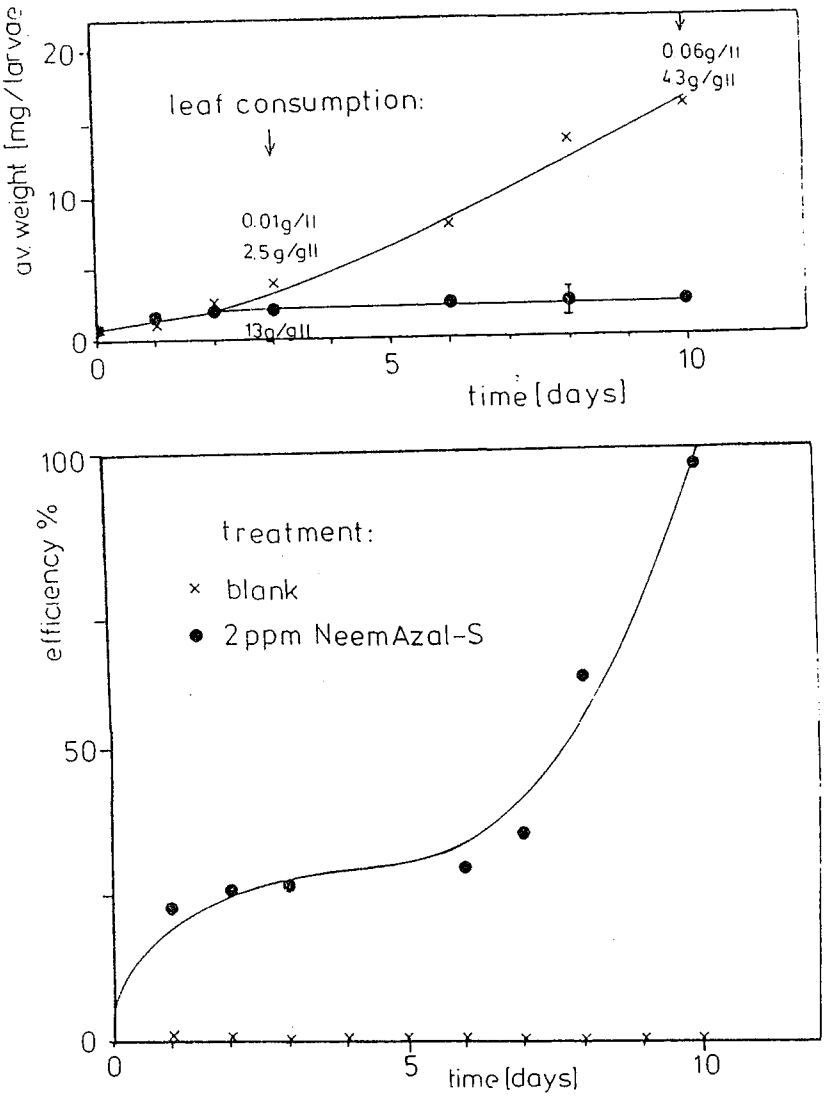


Figure 2: Change of the average body weight of living larvae *l* of Tenthredinidae after treatment with NeemAzal-S corresponding to 2 ppm Azadirachtin and with blank (upper part). The efficiency (mortality) of the treatment is indicated in the lower part of the figure. The larvae fed all the time of the experiment on the same leaves.

The Systemic Effect

In order to study the systemic effect of NeemAzal small twigs of a birch-tree were cut and fixed in a 150 ml glass with cardboard which covered the glass and which was fixed with adhesive tape to its top (see figure 3). The glasses were filled with tap water (control) and water containing NeemAzal-F corresponding to 10 and 100 ppm Azadirachtin, respectively. 10 Tenthredinidae larvae (probably *Croesus septentrionalis;* average weight: 33 mg/larva) were put on one leaf immediately afterwards and started feeding after some minutes.

The consumption of leaf mass was determined from the area of the digested parts of the leaves. Figure 4 shows the time-dependence of the leaf consumption after treatment in the upper part. After 8 days the control had consumed leaf mass corresponding to about 20 times their initial body weight. After about one day both

Figure 3: Setup for investigations of the systemic effect of NeemAzal-W.

The consumption of leaf mass was determined from the area of the digested parts of the leaves. Figure 4 shows the time-dependence of the leaf consumption after treatment in the upper part. After 8 days the control had consumed leaf mass corresponding to about 20 times their initial body weight. After about one day both sets of treated larvae stopped feeding and showed behavioural differences with respect to the control. After 3 days the larvae were different in length and colour: control: 16 mm, greenish; 10 ppm: 14 mm, yellowish; 100 ppm: 11 mm, yellowish. Both, the differences in colour and length seem to be due to the fact that the treated larvae stopped feeding. Until the 3rd day no larvae had died.

On the 2rd day the treated larvae had fed the first leaf totally, they started migrating and after some time some of them settled on the second leaf. However, they consumed only 1/5 or 1/2 of this leaf until the end of the test. On the control leaf consumption continued and pupation started at the fifth day. On the same day the first larvae had died on the treated twigs. 100% mortality was reached after 8 days.

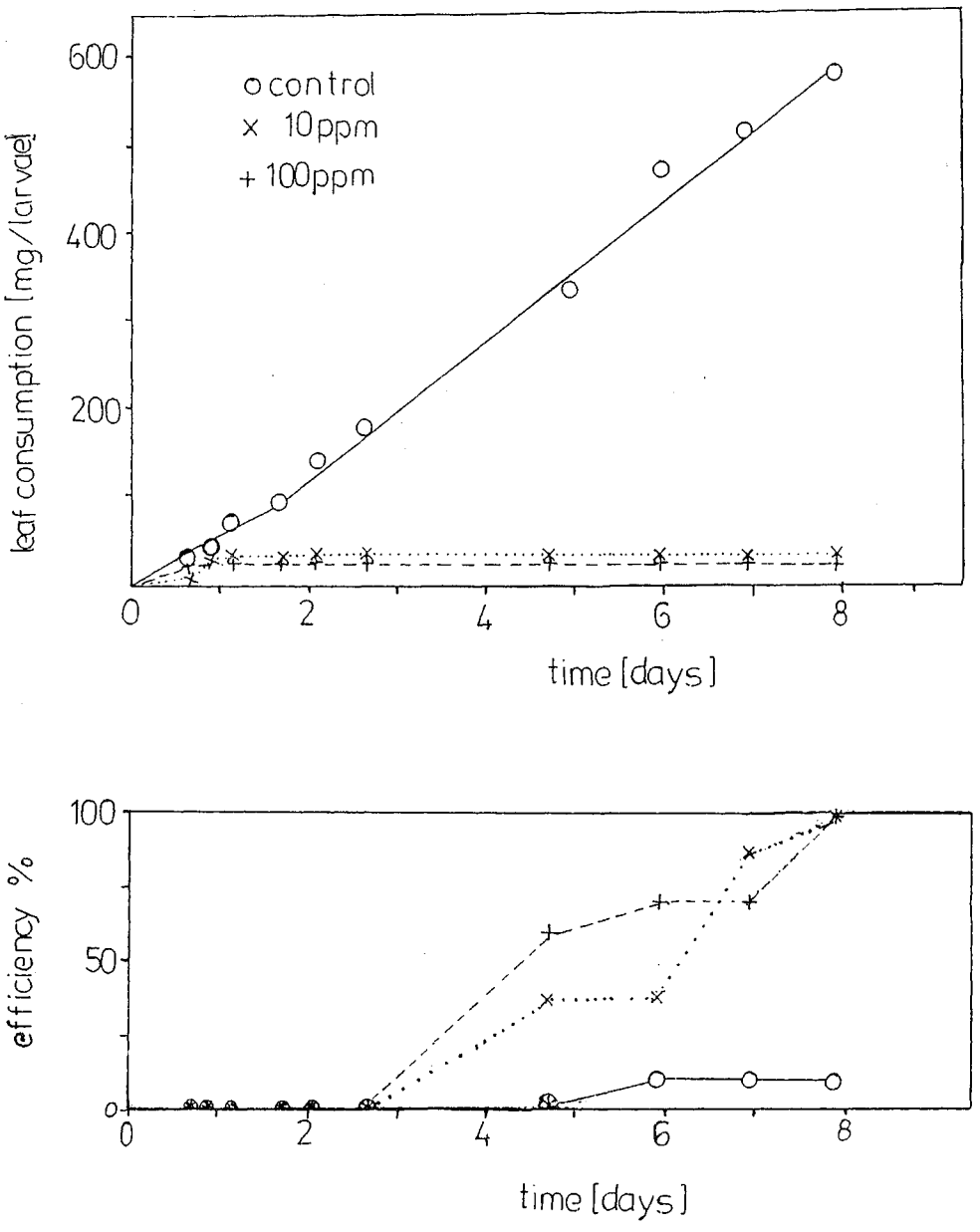


Figure 4: Leaf consumption of larvae on twigs in water (blank), or aqueous solution containing 10 or 100 ppm Azadirachtin (upper part). The efficiency (mortality) of the treatment is indicated in the lower part of the figure. The larvae fed all the time of the experiment on the same twigs.

Figure 5 illustrates the experimental situation after 6 days. In the control all leaves had been digested whereas only slightly more than one leaf had been touched in the NeemAzal-containing glasses.

Under the approximate assumption that the leaf mass contains the same amount of Azadirachtin as the aqueous solution with which the twigs are in contact it may be estimated that the larvae had taken up roughly 6 mg Azadirachtin per kg body weight after the first day. This order of magnitude agrees well with the lethal dose of Azadirachtin for *Pieris rapae* (Trifolio-M, 1989) and other pests.

Figure 5: Illustration of the experimental situation at the 6th day. Left: control, middle: 10 and right: 100 ppm Azadirachtin.

Comparison of figure 2 and 4 shows that the time dependence of the efficiency is very similar in the cases of spraying and systemic application of Azadirachtin containing solutions. From the comparison of leaf consumption and average weight of the larvae (upper parts of fig. 2 and 4), respectively, it may be concluded that - at least for the small twigs used in this investigation - the action of systemically transported Azadirachtin is at least as quick as after spraying, since clear effects between control and treatment are seen after 2 (see fig. 2) and 1 (see fig. 4) day, respectively.

In order to make a systemic application of NeemAzal possible we have to learn far more about the uptake of Azadirachtins by the roots of plants and its transportation through the plant. However, the systemic application of an insecticide may be a time saving, simple and user-friendly possibility for pest control - especially if irrigation systems are already available and have to be used, as for example in green houses.

Conclusions

All available information on active principles from Neem indicate that its usefulness for plant protection will be tremendous. Especially if plantation and afforestation programmes with Neem will increase, the availability of Neem seeds will make Neem-products available in sufficiently large scale.

Neem-insecticides may be one contribution to a really integrated approach in an environmentally sound pest management. Neem-ingredients, processed and formulated appropriately, will meet the highest future requirements of ecology and of safety for producers, farmers and consumers.

Trifolio-M invites interested parties for cooperation in order to improve the application technology of Neem pesticides with respect to the actual problems under different climatic conditions.

References

Böhmer B., Dalchow J., Gündel L., Krebs E.-K., Merz F., and Schickedanz F., 1991; Frankliniella - neue Erkenntnisse zur Bekämpfung, S. 807-812 Gärtnerbörse und Gartenwelt, Georgi & Parey, Aachen, Germany

Broughton H.B., Ley S.V., Slawin A.M.Z., Williams D.J., and Morgan E.D., 1986 Chem. Communs., p. 46-47

Dimetry N.Z. and Schmidt G.H., 1992, Efficacy of NeemAzal-S and Margosan-O against the bean aphid, *Aphis fabae* Scop., p. 75-79;Anz. Schädlingskde., Pflanzenschutz, Umweltschutz **65**, Verlag Paul Parey, Berlin

Gubler K., 1983, Innovation in Pesticide (Insecticide) Chemistry, in: Natural Products for Innovative Pest Management, p. 33-44 (D.L. Whitehead and W.S. Bowers (eds,)), Pergamon Press, Oxford, England

Horn D.J., 1988; Ecological Approach to Pest Management, Elsevier Applied Science Publ., London, England

Jacobson M. (ed.) 1989; Focus on Phytochemical Pesticides, Vol. 1, The Neem Tree, CRC Press, Inc., Boca Raton, Florida

Jones P.S., Ley S.V., Morgan E.D., and Santafianos D., 1989; in: Focus on Phytochemical Pesticides Vol. 1, The Neem Tree (Jacobson M. (ed.)) p. 19-45; CRC Press Inc., Boca Raton Florida

Karelina T.N., Filipov N.A., Kleeberg H., Kovalev B.G., and Puhalskya N.A., 1992
Evaluation of the Biological Activity of NeemAzal and NeemAzal-S against *Mamestra brassicae, Pieris rapae,* and *Heliothis armigera,* in: Insecticides - Mechanism of Action and Resistance (Otto D., ed.), Intercept Publ., Andover, England (in press)

Ketkar C.M. and Ketkar M.S., 1989; in: '89 Integrated Pest Management in Tropical and Subtropical Cropping Systems (DLG, ed.); p. 689-709; CTA, Ede, The Netherlands

Kleeberg H., 1992a; Properties of NeemAzal-F - A New Botanical Insecticide in: Insecticides - Mechanism of Action and Resistance (Otto D., ed.), Intercept Publ., Andover, England (in press)

Kleeberg H., 1992b; The Neem-Extractor; in: Insecticides - Mechanism of Action and Resistance (Otto D., ed.), Intercept Publ., Andover, England (in press)

Kleeberg H., 1992c, unpublished results

Menn J.J., 1983, Present Insecticides and Approaches to Discovery of Environmentally Acceptable Chemicals for Pest Management; p. 5-25, in: Natural Products for Innovative Pest Management (D.L. Whitehead and W.S. Bowers (eds,)), Pergamon Press, Oxford, England

National Research Council, 1992, Neem: A Tree For Solving Global Problems National Academy Press, Washington, D.C.

Schmutterer H., Ascher K.R.S., and Rembold H. (eds.) , 1980
Natural pesticides from the neem tree (Proceedings of the 1st Neem Conference) GTZ, Eschborn, Germany

Schmutterer H. and Ascher K.R.S. (eds.) , 1984
Natural pesticides from the neem tree and other tropical plants (Proceedings of the 2nd Neem Conference) GTZ, Eschborn, Germany

Schmutterer H., and Ascher K.R.S. (eds.) , 1988
Natural pesticides from the neem tree and other tropical plants (Proceedings of the 3rd Neem Conference, Nairobi) GTZ, Eschborn, Germany

Trifolio-M GmbH, 1989, The NeemAzal-Conception III, Lahnau, Germany

Erste Erfahrungen mit der Anwendung von NeemAzal-F

Edmund Hummel
Trifolio-M GmbH
Sonnenstr. 22
D-6335 Lahnau 2

EINLEITUNG:

Die zunehmende Anwendung von chemischen Mitteln belastet die Umwelt und schädigt die Gesundheit der Menschen. Daher wird es zunehmend notwendiger nach anderen umweltfreundlichen Alternativen Ausschau zu halten. Dies trifft in besonderer Weise für Pflanzenschutzmittel zu. Ein Dilemma von biologisch anbauenden Betrieben besteht darin, daß sie in gleicher Weise wie konventionell bewirtschaftete Betriebe dem Druck von "Schädlingen", "Unkräutern" und Krankheiten ausgesetzt sind, aber qualitativ und quantitativ nur unzureichende Mittel besitzen, um diesen Effekten gegenzusteuern.

Erfolgversprechende Präparate zur Bekämpfung von Schädlingen und Krankheiten könnten aus dem Neem-Baum gewonnen werden - wie beispielsweise das aus den Samen des Neem-Baumes gewonnene azadirachtinhaltige NeemAzal-F oder NeemAzal-S .

Die Firma Trifolio-M hat es sich zur Aufgabe gemacht, umweltfreundliche Pflanzenschutzmaßnahmen auf biologischer Basis (wie: botanische Insektizide und Fungizide, naturidentische Insektenlockstoffe, Nützlinge) zu entwickeln und deren Anwendung für die Praxis aufeinander abzustimmen.

In diesem Jahr wurden besonders Tests mit NeemAzal-F durchgeführt, an denen sich auch zahlreiche andere Institutionen und Interessenten beteiligten, denen wir dafür dankbar sind. Versuche wurden in verschiedenen Bereichen gegen Apfelwickler (Cydia pomonella), Apfelschalenwickler (Adoxophyes orana), Himbeerkäfer (Byturus

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

tomentosus), Erdbeerblütenstecher (Anthonomus rubi), Blattläuse im Gartenbau, den Rapserdfloh (Psylliodes ohrysocephala), Kartoffelkäfer (Leptinotarsa decemlineata), Spargelschädlinge (Crioceris-Arten) in Gemüse- und Ackerbau, gegen Traubenwickler im Weinbau sowie gegen den Gemeinen Schwammspinner (Porthetria dispar) im Forst durchgeführt.

Hierbei zeigte es sich, daß nicht alle Versuche mit NeemAzal-F auf Anhieb erfolgreich waren.

Erste Versuche mit Erdbeerblütenstechern und Himbeerkäfern :

In Versuchen mit Erdbeerblütenstechern (Anthonomus rubi) konnte bei der Bonitierung des Knospenbefalls keine deutliche Differenz zwischen der mit 0,2% NeemAzal-F behandelten Versuchsparzelle, der mit Quassia (0,2% Bionomic-Bitterholze Extrakt) behandelten Vergleichsparzelle (in beiden Fällen einmalige Behandlung zu Flugbeginn) sowie der unbehandelten Kontrollparzelle festgestellt werden. Der Knospenbefall in den Parzellen betrug 12.8%, 14,8% beziehungsweise 15,7%,

Auch die ersten Versuche mit Himbeerkäfern (Byturus tomentosus) schienen zunächst nicht sehr vielversprechend. Die beiden jeweils in zwei unterschiedlich stark befallenen Anlagen in 10-tägigem Abstand (am 15.5 und 25.5) durchgeführten Behandlungen mit 0.2% NeemAzal-F bzw. Quassia zeigten keine nennenswerte Verringerung der Schädigung von Blütenknospen im Vergleich zur nicht behandelten Kontrollparzelle (Bonitierung am 20., 25. und 29. 5).

Nach den Knospen befallen die Himbeerkäfer die grünen Früchte (Beeren). Bei der Bonitierung am 13. 6. wurde festgestellt, daß in beiden Anlagen die Früchte bei Versuchs-, Vergleichs- und Kontrollparzelle in ähnlichem Maße beschädigt waren.

Die am 3. 7. durchgeführte Ertragsbonitierung ergab in beiden Anlagen einen deutlich geringeren Schädlingsbefall der reifen Früchte in der Versuchsparzelle im Vergleich zu der mit Quassia behandelten und der nicht behandelten Parzelle (s. Abb. 1). Bei den beiden Sorten Schönemann und Zewa waren nach NeemAzal-Behandlung 21 bzw. 12% befallen, während im Vergleichsversuch 35 und 20% und ohne Behandlung 42 und 27% Larven des Himbeerkäfers aufwiesen (s. Abb. 1).

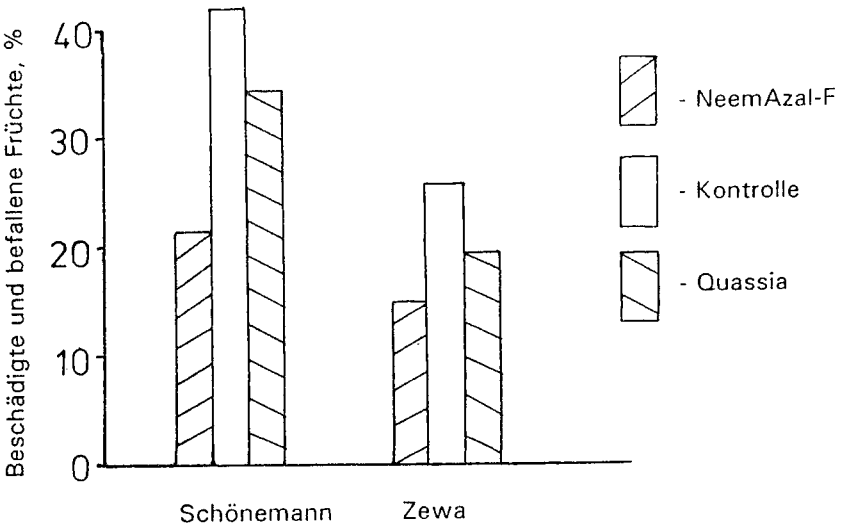


Abbildung 1: Bei Ertragsbonitierung am 3. Juli bestimmte Anteile beschädigter und befallener reifer Himbeerfrüchte nach Behandlung mit 0,2% NeemAzal-F, 0.2% Quassia und ohne Behandlung.

Aufgrund der Ergebnisse der Qualitätsbonitierung kann vermutet werden, daß die Himbeerkäfer, die die mit NeemAzal behandelten Knospen beschädigt haben, später weniger Eier abgelegt haben als die Käfer der Vergleichs- oder Kontrollparzelle.

Weitere Versuche mit Erdbeerblütenstechern und Himbeerkäfern mit besonderem Augenmerk auf eine Optimierung des Behandlungstermins und gegebenenfalls unter Verwendung höherer Wirkstoffkonzentrationen müssen zeigen, ob eine wirtschaftlich sinnvolle Anwendung von Neem-Azal-F möglich sein wird.

Versuche mit Kartoffelkäfern:

In einem Vorversuch wurden 3 Kartoffelpflanzen (Sorte "Christa") am 4. 6. mit 0.5% NeemAzal-F behandelt. Die Kartoffelkäfer (Leptinotarsa decemlineata) befanden sich zu diesem Zeitpunkt im I. und II. Larvenstadium. Nach vier Tagen (s. Abb. 2) war der Befall bei den Versuchspflanzen auf 3 bis 33% gesunken, während er bei den beiden Vergleichspflanzen noch 61 bis 83% des Wertes vor der Behandlung betrug. Die Verringerung der Schädlingspopulation bei den Kontrollpflanzen und ein Teil der Verringerung bei den Testpflanzen ist vermutlich auf starke Regenfälle und/oder Nützlinge zurückzuführen.

Bei Bonitierung am 12. 6. (s. Abb. 2) betrug der Schädlingsbefall der Testpflanzen 0, 0 bzw. 33%, wobei die Larven der dritten Pflanze wesentlich in ihrer Entwicklung gegenüber denjenigen der Kontrolle zurückgeblieben waren. Auf der Testpflanze hatten lediglich 5% der Larven das IV. Stadium erreicht und 28% waren im II. und III. Larvenstadium, während auf der Kontrolle alle Larven das IV. Stadium erreicht hatten. Am 15. 6. hatten alle Larven die Kontrollpflanzen verlassen, um sich im Boden zu verpuppen. Bei der 3. Versuchspflanze konnten zum gleichen Zeitpunkt noch 12% Larven in den Entwicklungsstadien II bis IV gezählt werden.

Die Wiederholung dieses Versuches mit unbehandelten Kartoffelpflanzen (alle Schädlinge im I. Larvenstadium) am 8. 6. zeigt ähnliche Ergebnisse (s. Abb. 3, linker Teil). 4 Tage nach der Behandlung wurden auf den Testpflanzen keine lebenden Larven mehr gefunden. Der geringfügige Anstieg der Schädlingspopulation (ausschließlich I. Larvenstadium) am 15. 6. auf Testpflanze 1 wird mit dem erneuten Schlüpfen von Larven aus der Eiablage, die nicht hinreichend mit NeemAzal-F benetzt wurden, erklärt.

Auch die Behandlung mit 0.3% NeemAzal-F (Versuch vom 12. 6.; II. und III. Larvenstadium; s. Abb. 3, rechts) zeigt entsprechende Ergebnisse: 3 Tage nach der Behandlung waren auf den Testpflanzen alle Larven im II und III Larvenstadium tot; die 22% der Larven im IV. Stadium auf Pflanze 3 waren deutlich weniger aktiv.

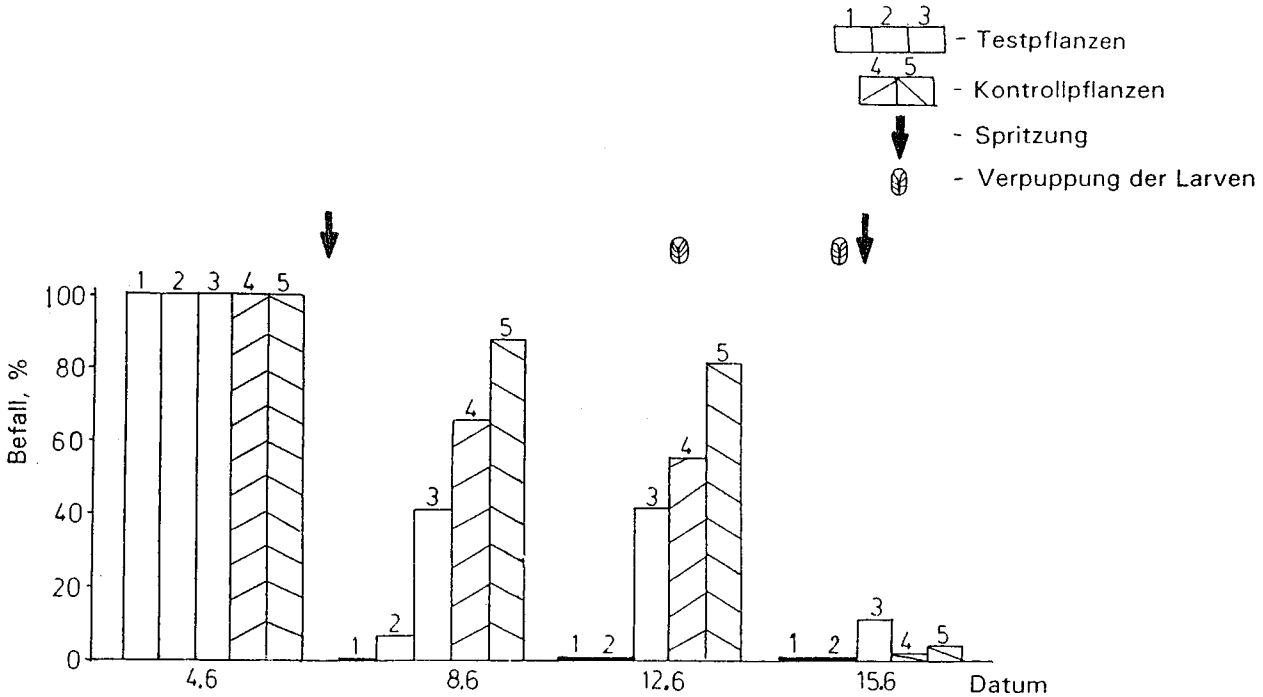


Abbildung 2: Befall der Kartoffelpflanzen mit Kartoffelkäfern nach Behandlung mit 0.5% NeemAzal-F.

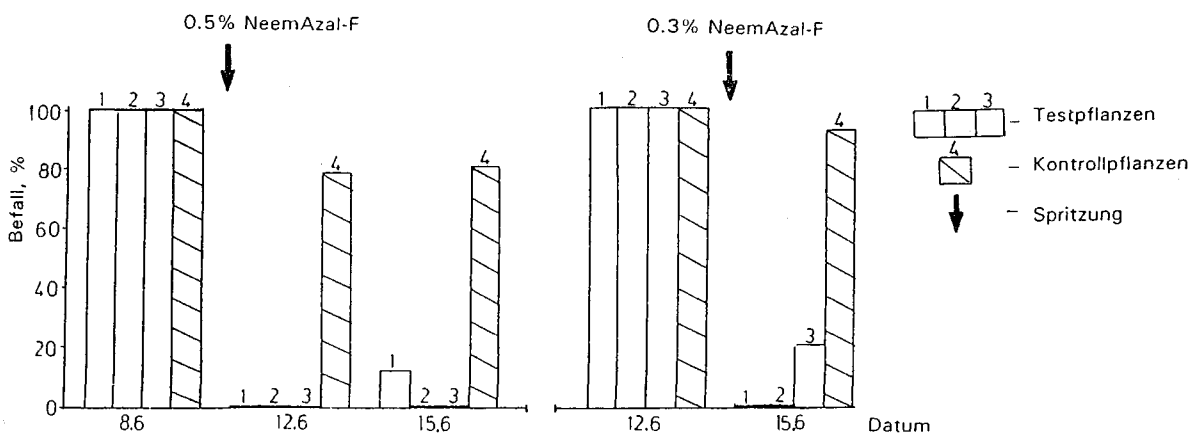


Abbildung 3: Befall der Kartoffelpflanzen mit Kartoffelkäfern nach Behandlung mit 0.5 (linker Teil) und 0.3 % NeemAzal-F (rechter Teil).

Schlußbetrachtung:

Aufgrund der bisherigen eigenen Erfahrungen können wir feststellen, daß NeemAzal-F eine sehr gute Wirksamkeit gegen beißende Schädlinge wie Kartoffelkäfer, Kohlweißlinge, Blattwespen- und Eulen-Arten besitzt. Bei einigen Schädlingen (siehe Versuche mit Erdbeerblütenstechern und Himbeerkäfer) ist die Terminierung der Anwendung noch genau zu prüfen, bevor entschieden wird, ob ein großflächiger Einsatz von NeemAzal-F sinnvoll ist. Es wurde allgemein beobachtet, daß die Anwendung von NeemAzal-F auf junge Larvenstadien deutlich geringere Konzentrationen erfordert als bei älteren Larven. Daher wird der Schwerpunkt unserer weiteren Untersuchungen in der Optimierung von Anwendungszeitpunkt und Anwendungskonzentration liegen.

Die Anwendungsmöglichkeiten des Präparates NeemAzal-F in GUS-Ländern

B. G. Kovalev, I.A. Nesterov, T.N. Karelina, und H. Kleeberg
Institut der Wissenschaftsakademie der Modova-Republik für
Biologische Methoden im Pflanzenschutz
Prospect Mira 58, 277072 Kishinev 72
MOLDOVA

Die Entwicklung von botanischen Insektiziden ist die vordringlichste Aufgabe innerhalb von Umweltschutzmaßnahmen zur Verbesserung der Lebensgrundlagen und damit der Gesundheit der Menschen.

Azadirachtin ist in diesem Sinne ein ideales Päparat (hohe biologische Aktivität gegen Schädlinge; geringe Toxizität für Warmblütler und Nützlinge; hinreichende Stabilität des Wirkstoffes bei leichter biologischer Abbaubarkeit; Moleküle beinhalten lediglich Kohlenstoff, Wasserstoff und Sauerstoff aber keine Heteroatome).

Zwischen 1990 und 1992 wurden von uns einige Substanzen, die auf Azadirachtinbasis beruhen (NeemAzal, NeemAzal-S, NeemAzal-F), im Labormaßstab gegen blattfressende Schädlinge getestet, wie: Kohlschädlinge: Mamestra brassicae und Pieris rapae, Baumwollkapselwurm (Heliothis armigera), Amerikanischer Webebär (Hyphantria cunea), Bekreuzter Trauben-wickler (Lobesia botrana), Kartoffelkäfer (Leptinotarsa decemlineata).

Diese Tests haben die hohe biologische Aktivität dieser von uns untersuchten Substanzen gezeigt, wobei 0,0002 bis 1,0%-ige Anwendungen in Abhängigkeit von Larvenstadien und der gewählten Formulierung untersucht wurden.

Dabei wurden verschiedene (juvenoide, insektizide und deterrente) Aspekte der Wirkungsaktivität von Azadirachtin festgestellt (s.: Karelina T.N., Filippov N.A., Kleeberg H., Kovalev B.G., and Puhalskya N.A., Evaluation of the Biological Activity of NeemAzal and NeemAzal-S against Mamestra brassicae, Pieris rapae, and Heliothis armigera, in: Insecticides - Mechanism of Action and Resistance, Otto et al. (eds.), Intercept Publ., Hampshire (im Druck)).

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Zwischen 1991 und 1992 haben wir Feldversuche zur Entwicklung der Anwendungstechnologie von NeemAzal-F gegen Kartoffelkäfer (Leptinotarsa decemlineata) auf Kartoffeln und Auberginen und gegen den bekreuzten Traubenwickler (Lobesia botrana) begonnen.

1991 wurde eine 150 m^2 große Kartoffelparzelle mit 0,1 und 1% NeemAzal-F behandelt. Als Kontrolle diente eine unbehandelte Parzelle und als Vergleich eine mit 0.1% Decis behandelte Parzelle.

Die Ergebnisse sind in der folgenden Tabelle dargestellt.

Tabelle: Die Wirkung von NeemAzal-F auf die Larven des Kartoffelkäfers bei Behandlung der Kartoffelpflanzen.

Variante	Konzentration	Anzahl der Larven auf einer Pflanze in % nach: 2	5	10 Tagen	Schädigung der Pflanzen (%) nach 10 Tagen
NeemAzal-F:	1,0	72,3±0,9	32,3±1,3	41,2±0,9	11,5±1,2
	0,1	74,4±1,1	40,3±0,7	41,8±0,9	14,7±1,3
Decis	0,1	17.6±1,2	25,5±1,3	41,3±1,0	16,0±0,9
Kontrolle	-	100,0±0,5	117,0±1,1	132,0±1,2	99,3±0,4

Bei Versuchsbeginn waren auf jeder Pflanze im Durchschnitt 52 Larven. Im Gegensatz zur Vergleichsparzelle, in der Decis eingesetzt wurde, haben wir in der Versuchsparzelle erst am 5. Tag tote Larven gefunden, während sich in derselben Zeit die Schädlinge in der Kontrollparzelle auf 117 % vermehrt hatten (s. Tabelle). Gleichzeitig wurde in allen Parzellen das Schlüpfen der neuen Larven aus der Eiablage beobachtet. NeemAzal-F und Decis zeigten dabei keine ovizide Wirkung. Obwohl die Larvenanzahl in der Versuchsparzelle deutlich höher war als im Vergleich, war der Zustand der Pflanzen nach 10 Tagen nicht schlechter. Dies erklärt sich aus der Beobachtung, daß die geschädigten Larven fast nichts gefressen und sich daher auch nicht weiterentwickelt haben.

Abbildung: Kartoffeln mit Kraut aus der Kontrollparzelle (links) und den beiden mit 0.1 (mitten) und 1% (rechts) NeemAzal-F behandelten Versuchsparzellen.

Zu diesem Zeitpunkt waren die potentiellen Schädlinge die nach der Behandlung geschlüpften Larven. Daher wurde am 10. Tag - bei überwiegendem Befall der Versuchsparzelle mit Larven im 2. Stadium - die 2. Behandlung durchgeführt. Damit war ein guter Ertrag der Versuchsparzelle gesichert (s. Abbildung).

In der Kontrollparzelle wurden die Blätter der Pflanzen von den Larven, die in ihrer Entwicklung nicht gehindert wurden, stark geschädigt, bevor die Larven zur Verpuppung in den Boden wanderten (s. Tabelle).

Die Versuche 1992 scheinen zu vergleichbaren Ergebnissen zu führen. Es wurde zudem festgestellt, daß NeemAzal-F in der Konzentration 0,05 % auch mit Erfolg gegen Larven im 2. und 3. Stadium eingesetzt werden konnte.

Die Behandlung von 2 ha Traubenanlagen mit NeemAzal-F, in 0,05%iger Konzentration gegen die Raupen von Lobesia botrana, ergab nach 5 Tagen eine Reduzierung der Raupenzahl von 12 pro 100 Fruchtstände auf 2,6 pro 100 Fruchtstände. Im Kontrollfeld hat sich die Anzahl der Raupen kaum verändert (10 Raupen pro 100 Fruchtstände vor und 10,7 Raupen pro 100 Fruchtstände nach der Behandlung).

Wir werden die Versuche zur Optimierung der Anwendungstechnologie von NeemAzal-F weiter fortsetzen.

EFFECTS OF NEEMAZAL-F ON APHIDS AND BENEFITIAL INSECTS IN PEACH ORCHARDS IN FRANCE

Eisenlohr U. *, Domange A.L.*, Lenfant C.**, Sauphanor B.**

* G.R.A.B. (Groupe de Recherche en Agriculture Biologique) Agroparc - B.P. 131 - 84146 MONTFAVET - FRANCE

** I.N.R.A. (Institut National de Recherches Agronomiques) Station de Zoologie, Apidologie, Domaine St. Paul - 85143 MONTFAVET FRANCE

Aphids are important pests in fruit culture in France. In organic culture, there isn't yet any satisfactory means of control.

We have tested the effect of a neem (Azadirachta indica) formulation, the NeemAzal-F, against the green peach aphid (Myzus persicae), which is a principal pest in peach orchards.

The study consists of 2 parts:
- a field experiment
- laboratory tests

Field experiment (G.R.A.B.)
- Effect of NeemAzal-F on Myzus persicae compared with Biophytoz (another natural insecticide, containing pyrethrum and rotenon).
- Countings of beneficial insects on the different treatments.

Laboratory tests (I.N.R.A)
Effect of NeemAzal-F on:
- Myzus persicae (at different concentrations) (preliminary test)
- the development of beneficial insects found in trap bands during the field experiment
- the common earwig (Forficula auricularia) (3 experiments)
- the oriental peach moth (Cydia molesta): (3 experiments)

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

PRELIMINARY TEST

Before starting the field experiment, we made a preliminary test in the laboratory, to estimate the effect of NeemAzal-F on Myzus persicae and to determinate the concentrations of the treatment.

There were 4 treatments: - a control treated with water
- NeemAzal-F at 10 ppm, 50 ppm, 250 ppm

For each treatment 12 first instar larvae were put on 12 shoots of peach plants after the treatment had dried. Treatments were carried out with a hand sprayer.

Figure 1 shows the mortality of the larvae. The treatment had no effect at 10 ppm. Treatments at 50 an 250 ppm were both effective. Both treatments caused complete mortality (10 days after treatment at 50 ppm and one day later at 250 ppm). At 50 ppm, the treatment showed its effect later than at 250 ppm. There was a high mortality rate at the final moult at 50 ppm (5 days after treatment) and already 2 days after treatment at 250 ppm.

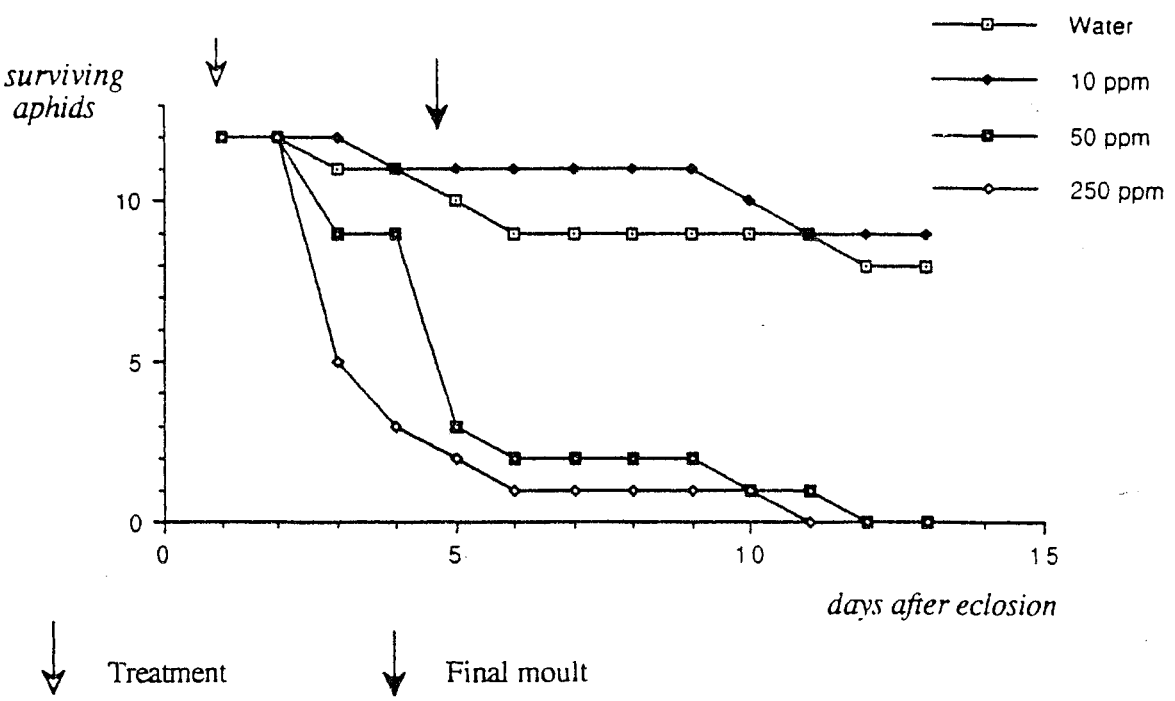


Figure 1: Sensitivity of M. persicae to NeemAzal-F.

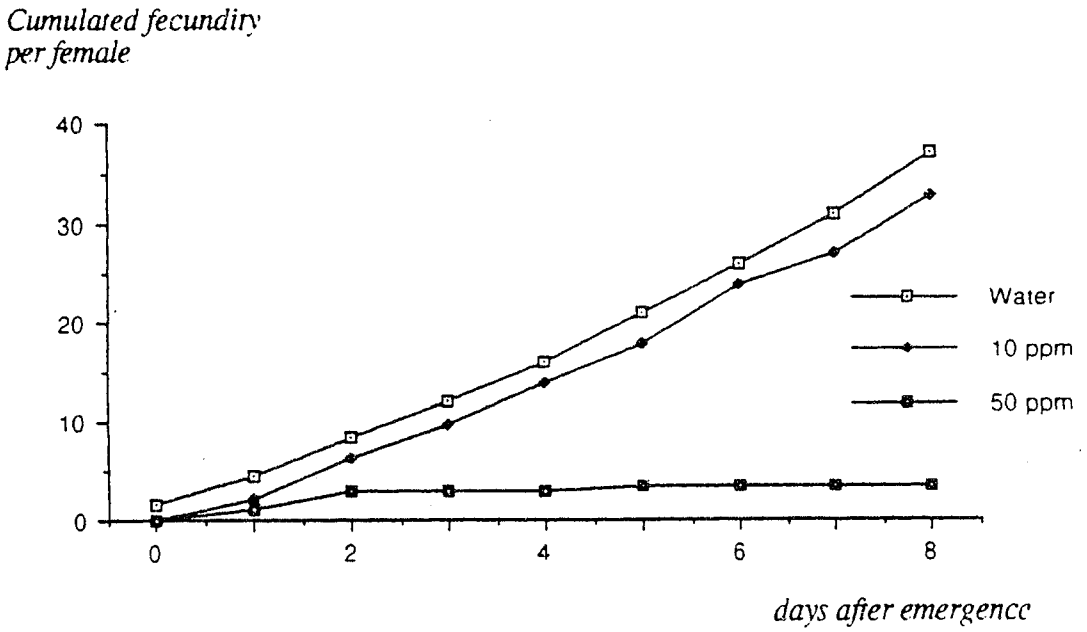


Figure 2: Fecundity of females of M. persicae raised on NeemAzal-F treated peach plants.

Figure 2 shows the influence of the treatment on the females emerging from the larvae which had been raised on the treated plants.

The fecundity of the females on the 10 ppm treatment was retarded for 24 hours, but later developed as much as on the control. There was no more fecundity on the 50 ppm treatment after the second day after the emergence of the females. As a consequence of these laboratory results, we chose a concentration of 50 ppm for the field experiment.

FIELD EXPERIMENT

Materials and methods

4 treatments :
- NeemAzal-F (1 application)
- NeemAzal-F (2 application)
- Biophytoz L (1 application)
- control

Name of commercial product	Active Ingredient	Concentration of active ingr. comm. product	Concentration at spraying of	
			commercial product	active ingredient
NeemAzal-F	Azadi-rachtin	5,0 %	0,1 % NeemAzal-F	50 ppm Azadirachtin
Biophytoz L	pyrethrum rotenon	1,5 % 3,0 %	0,35 % Biophyttoz L	52,5 ppm pyrethrum 105,0 ppm rotenon

The treatments were carried out at 1200 l pro ha with a knapsacksprayer.

The first treatments were carried out on the April 22., the second treatment (NeemAzal-F) 13 days later (5. May). There were 5 replications per treatment, each replication consists of a plot of 4 trees.

Methods of control

1 - Control of the infestation of the whole tree:
We counted the number of infested shoots on the two central trees of esch plot for 2 minutes per tree (10 trees per treatment).

2 - Estimation of the development of the colonies
We chose 10 infested shoots on the two middle trees of each plot (50 shoots per treatment).

The size of the colonies was estimated in 5 classes:

- class 1 : 0 - 5 aphids per shoot
- class 2 : 5 - 10 " " "
- class 3 : 10 - 20 " " "
- class 4 : 20 - 50 " " "
- class 5 : 50 " " "

3 - Beneficial insects

We counted the number of syrphid and ladybird oviposition and larvae at each control on these 50 shoots per treatment.

Both types of control were carried out on 5 different dates:

21.4. : 1 day before treatment
24.4. : 2 days after "
29.4. : 7 " " "
6.5. : 14 " " "
12.5. : 20 " " "

Results of the field experiment

Figure 3 shows the evolution of the infestation on the whole tree.

Because the number of infested shoots was very different between the treatments at the beginning of the experiment, we calculated the values for the same point of departure. The level of infestation shows the percentage of infested shoots of the initial value 100.

The level of infestation decreases on all treatments until the second day after treatment and then remains at nearly the same level for about a week. Later, the infestation increases on all treatments.

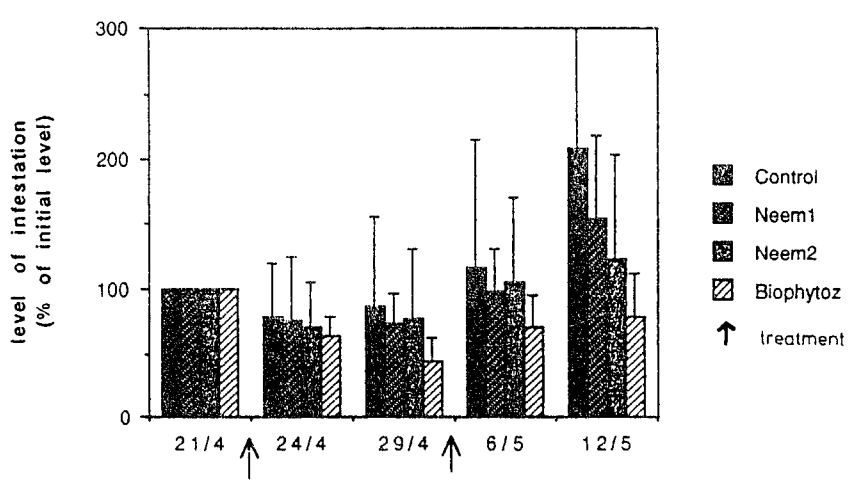


Figure 3: Evolution of infestation on 10 trees per treatment (each tree has been controled during 2 minutes).

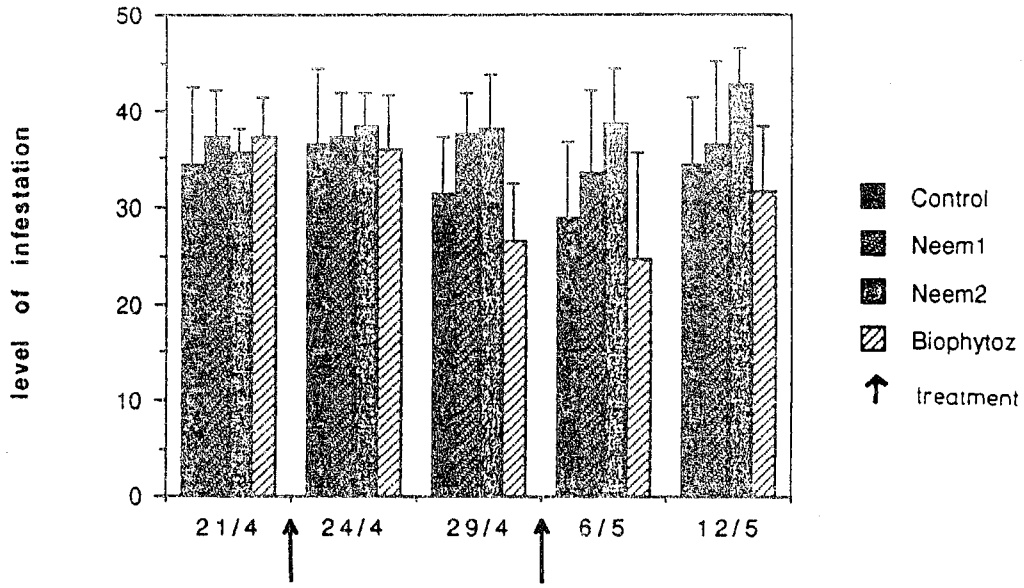


Figure 4: Effect of Neem on M. persicae in PEACH ORCHARD:
Evolution of the infestation on 50 shoots per treatment.

There is no difference between the evolution of the control and that of the NeemAzal-treatment.

The Biophytoz-treatment causes a slight decrease in the infestation compared to the control (and prevents a strong increase in the infestation). A more precise analysis is not possible because of the high variability of the standard deviation.

Figure 4 shows the effect of NeemAzal-F on Myzus persicae estimated by the control of 50 shoots per treatment.

To obtain the level of infestation we calculated the average of the cumulated class-indices. This means that the highest value obtainable is 50.

The number of aphids on the control decreases slightly until two weeks after treatment. There is a slight decrease on the Biophytoz-treatment compared to the control. But the infestation on both Neem modalities remains at about the initial level until 2 weeks after treatment.

There is a slight increase on all treatments at the 12. 5. (20 days after treatment).

With regard to both controls we could not observe a difference between the NeemAzal-F treated plots and the control and only a slight decrease of the infestation on the Biophytoz-treatment compared to the control.

CONTROL

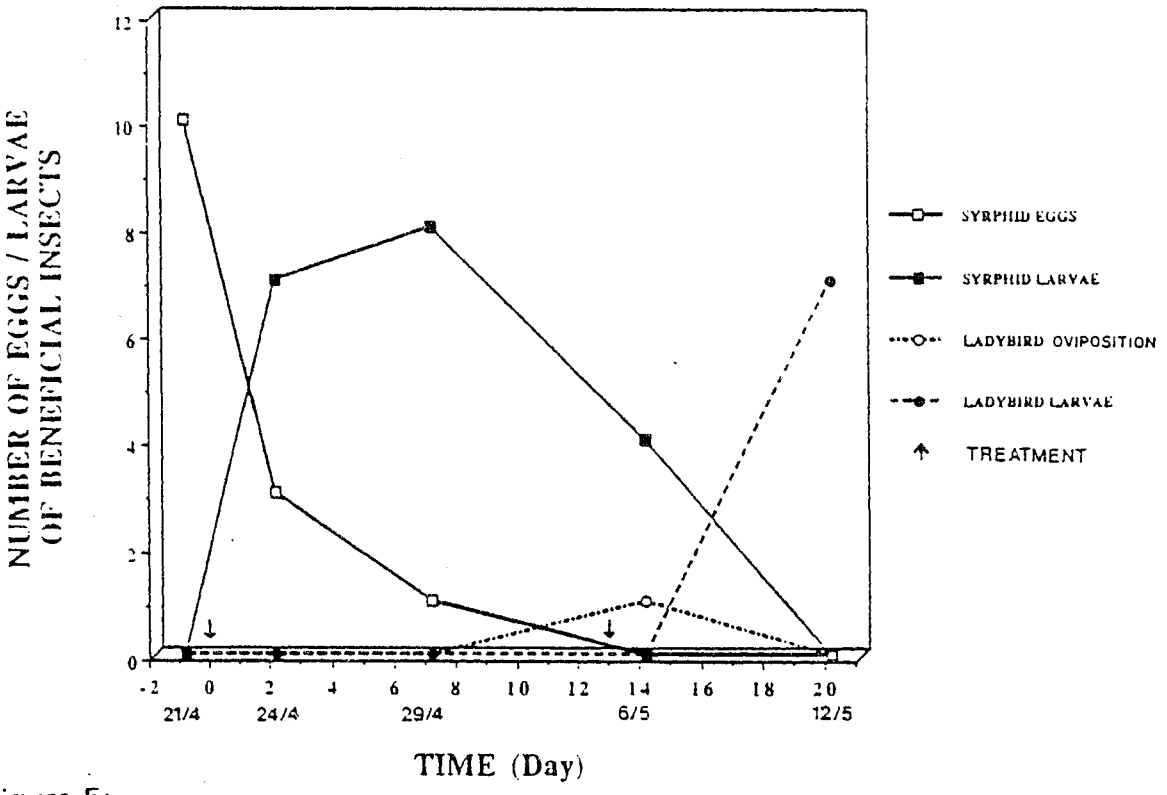


Figure 5:

BIOPHYTOZ

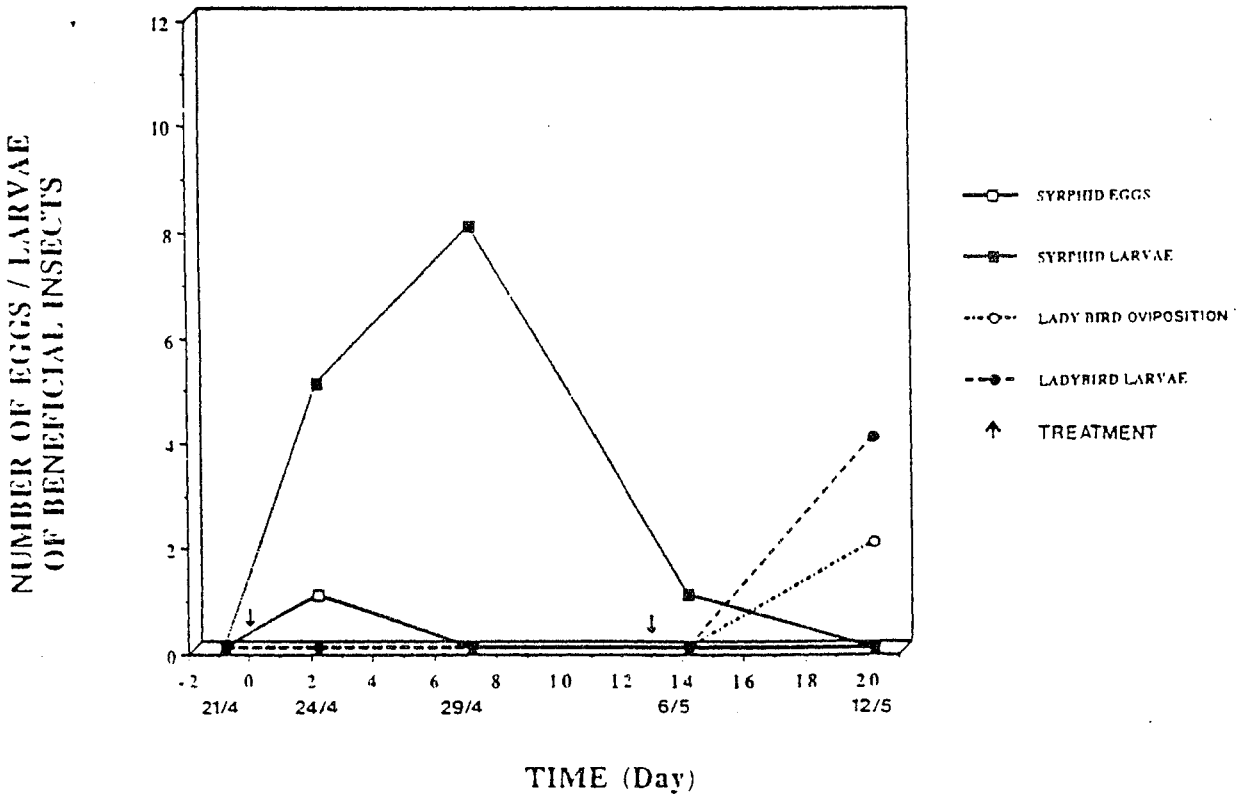


Figure 6:

NEEM 1

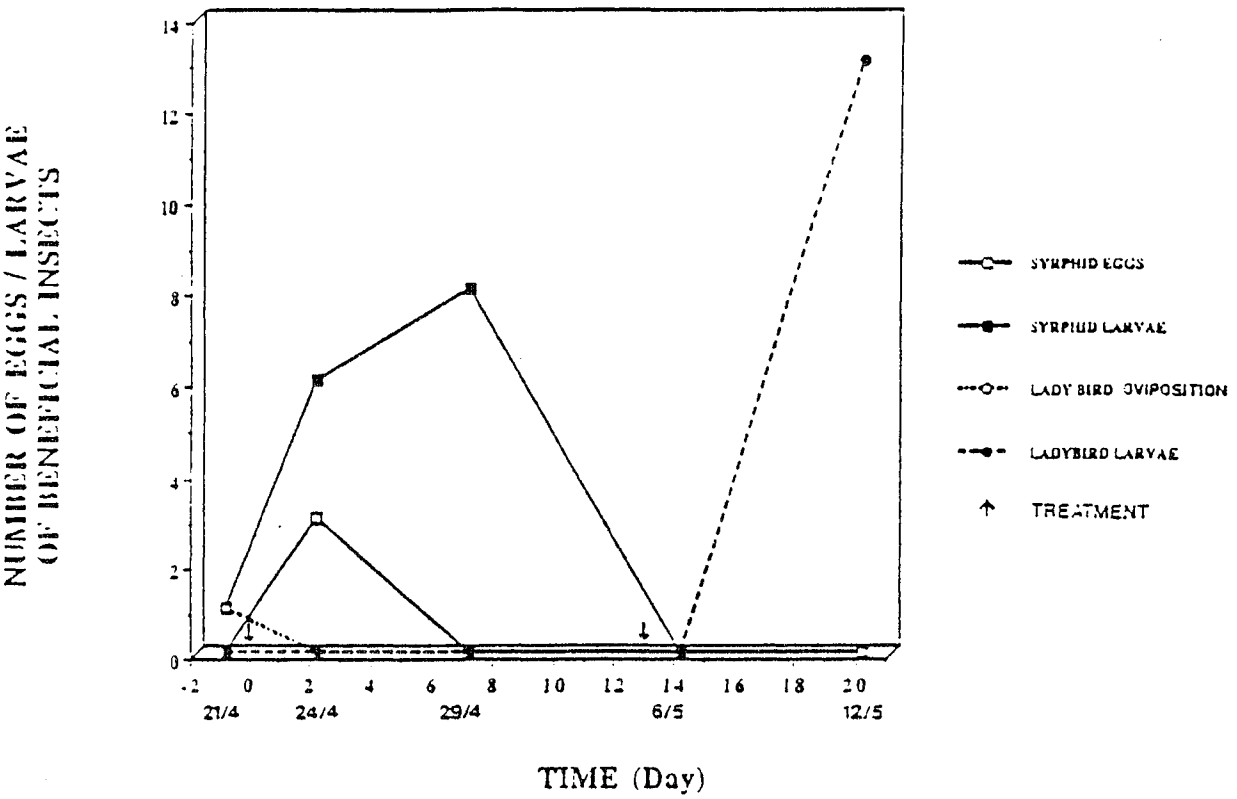


Figure 7:

NEEM 2

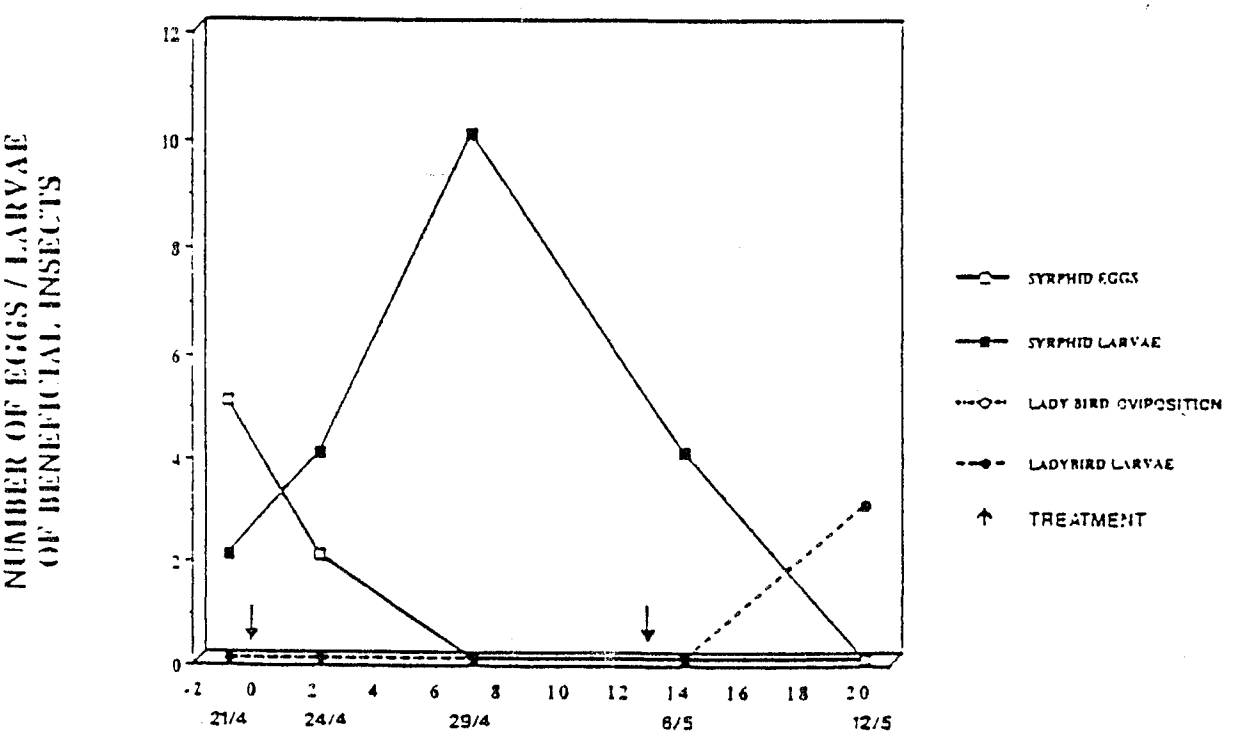


Figure 8:

The results on beneficial insects are shown in figures 5 to 8.

We counted oviposition and larvae of syrphs and ladybirds on 50 shoots per treatment. Syrphid and ladybird larvae are important predators of aphids (Lyon and Göldin de Tiefenau, 1974).

On the 21. 4. (1 day before treatment) we observed the highest number of syrphid eggs and already few syrphid larvae. The number of syrphid larvae increased until the 29.4 (one week after treatment), when we found the highest number on all the treatments. The last larvae were counted one week later, where we also found the first pupae.

On the 12. 5. (20 days after treatment) the first ladybird larvae hatched from the eggs. This development is about the same for all treatments.There was no difference between the total number of syrphid larvae on the different treatments.

We could not observe a negative effect of NeemAzal-F on the beneficial insects by this simple method of control.

LABORATORY TESTS

1 - Effect of NeemAzal-F on beneficial insects found in trap bands

We compared the number of syrphid, earwig larvae and ladybird oviposition found in trap bands fixed at the stems of the two middle trees of the plots. The common earwig is also considered as a predator of aphids (Lenfant and Sauphanor, 1992).

There were 2 captures of syrphid larvae and nymphs, 1 capture of ladybird oviposition and 2 captures of common-earwig larvae, (on 9 NeemAzal-F treated plots, 5 Biophytoz-treated and 5 untreated plots). The larvae found in the trap bands were raised in laboratory to follow their development.

Table 1 : Results of Syrphid larvae.

	Syrphs 24. 4. (2 days after treatment)		Syrphs 4. 5. (12 days after treatment)	
	captures/tree (larvae and nymphs)	% of emergence (15. 5.)	captures/tree (larvae and nymphs)	% of emergence (18.5.)
control	1,9	52,6	3,4	82,4
Biophytoz	1,6	37,5	5,3	54,7
NeemAzal	1,6	27,6	3,5	30,2

We could not observe a reduction of the number of syrphid larvae by the different treatments, compared to the control.

But the number of syrphid adults hatching from the pupae was lower than in the control with those found on the Biophytoz-treated plots and even lower in the NeemAzal-treated plots (on both takings).

Table 2 : Ladybird oviposition, 4. 5. (12 days after treatment).

	captures/tree
control	3,9
Biophytoz	2,0
NeemAzal	4,1

There was no difference between the oviposition of ladybird on the control and the NeemAzal-treatment. But there was only half of the oviposition on the Biophytoz-treatment.

Table 3 : Results of the common earwig.

	Common earwig, 29. 4. 7 days after treatment		Earwig, 4. 5. 12 days after treatment	
	29. 4. capt./tree	5. 6. survival %	4. 5. capt./tree	5. 6. survival %
control	1,4	100	4,7	83
Biophytoz	0,8	100	3,0	83
NeemAzal	0,4	86	1,7	90

One week after treatment only 57 % of the number of earwig larvae were found on the Biophytoz-treatment (compared to the control) and only 29 % on the NeemAzal-treatment. 5 days later we found 64 % of the larvae on Biophytoz (compared to the control) and only 36 % on NeemAzal. However, the development of the larvae was not affected by both treatments.

2 - Effect of NeemAzal-F on the larval development of the common earwig

In the first test NeemAzal-F was introduced in an artificial diet at concentrations of 25, 50 and 250 ppm. There were 5 replications per treatment with 10 larvae per replication. At the beginning of the test, the larvae were in the second instar. Every 3 days the food was changed and its weight recorded. Weight, phenology and mortality of the insects were recorded.

Figure 9 shows that there is a strong reduction of the consumption on all treatments compared to the control.

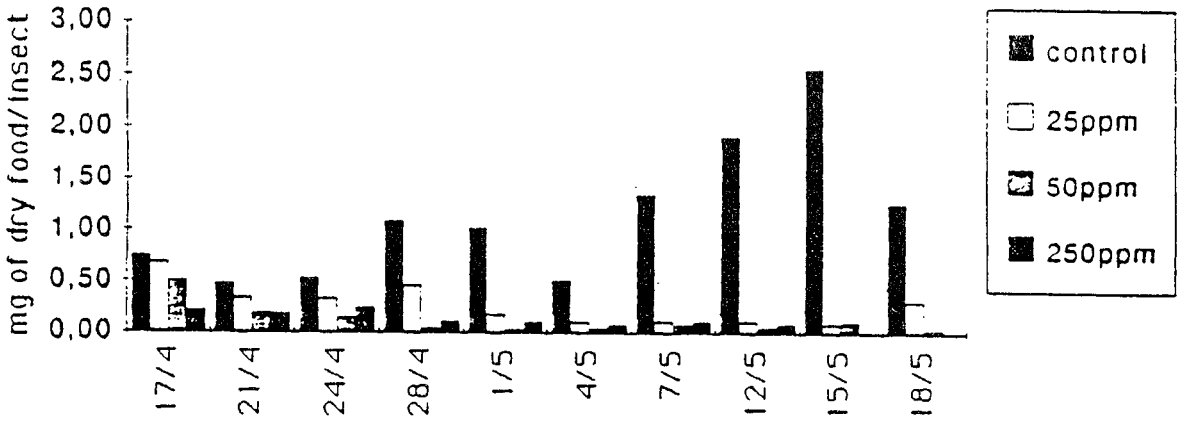


Figure 9: Evolution of the daily consumption during the larval development.

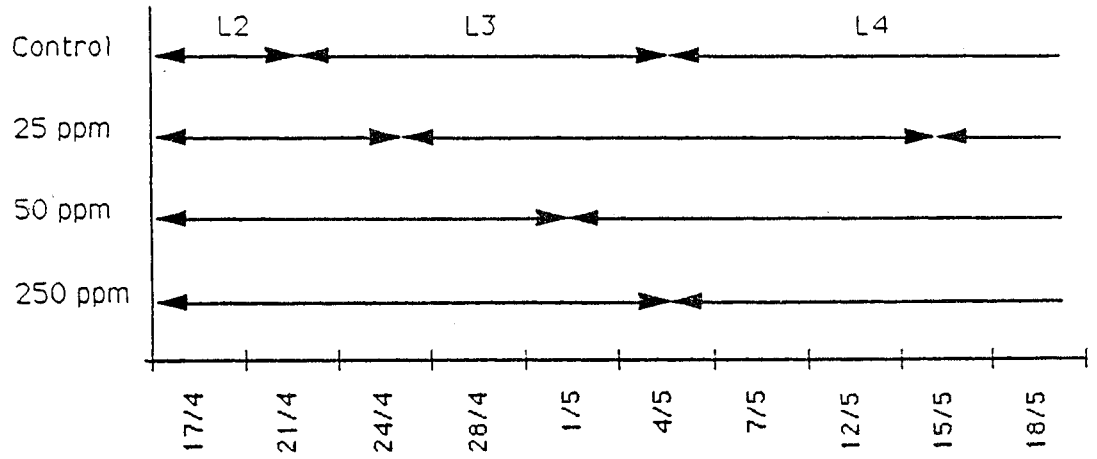


Figure 10: Delay in the larval development (limits indicate that 50% of the insects of the following instar had emerged).

Figure 10 shows the delay in the larval development with the treated diet compared to the control. The delay increases with the concentration.

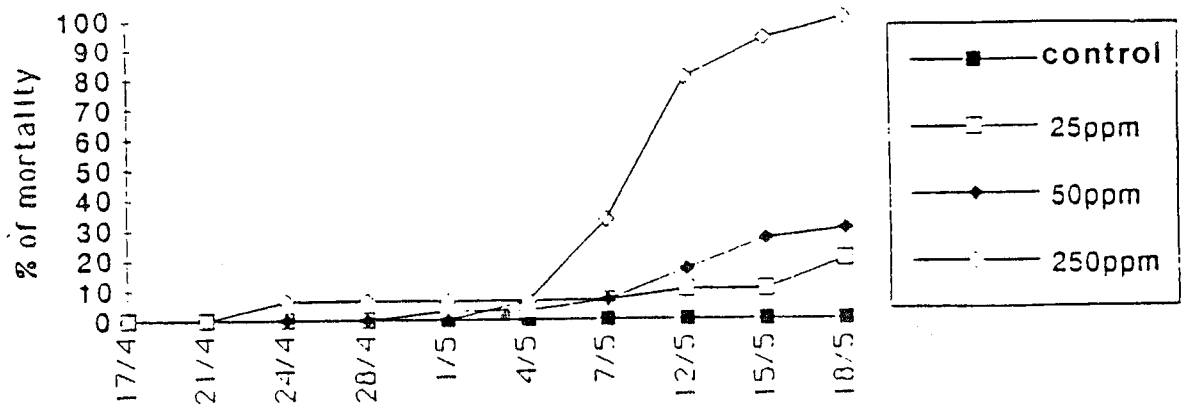


Figure 11: Evolution of the mortality during the larval development.

The mortality of the larvae is shown on Figure 11.

There is no mortality of the control, a low mortality at 25 and 50 ppm and 100 % mortality at 250 ppm NeemAzal-F treatment, one month after the beginning of the experiment. There is no direct toxicity. The mortality is caused by not feeding.

In the second test NeemAzal-F was incorporated in an artificial diet at a concentration of 50 ppm. Treated and control food was given to the insects in the course of a choice situation. There were 10 replications with 10 insects per replication.

The consumption was recorded after 3 days. There was no food preference.

Another test was made to find out if there is a repellent effect:
In the third test small pieces of cardboard were soaked in a NeemAzal-F solution of 50 ppm or in water. Treated or control shelters were given to the insects in course of a choice situation. There were 30 replications, with respect of the photoperiod. After one hour, the occupation of each type of shelter was recorded.
No repellent effect could be observed.

3 - Oriental peach moth

NeemAzal-F was incorporeted in the artificial diet of neonate larvae of the oriental peach moth (Cydia molesta). There were 8 replications per treatment with 20 larvae per replication. Three different concentrations of NeemAzal-F were compared with a control.

Table 4 shows that there was a higher mortality with the treated diet than on the control (24 hours after beginning of the experiment). The different concentrations had about the same effect. There were no adults emerging on all of the Neem treatments compared with 22,5 % mortality on the control.

In the second and third tests (table 5 and 6), peach fruit were hung up and infested with neonate larvae of Cydia molesta after treatment with NeemAzal-F at different concentrations (10 larvae per 10 fruits per treatment). The total survival was significantly lower on the treated fruit than on the control (table 5). There was a mortality rate of 50 % at a concentration of 50 ppm.

These results could be confirmed by the third test (table 6). There was no higher mortality at 100 ppm.

Table 4 : Incorporation of NeemAzal-F in artifical diet:
(20 neonate larvae x 8 replications)/treatment.

	Control	25 ppm	50 ppm	100 ppm
mortality	11,3	18,8	25	36,3
mortality before final moult	22,5	100	100	100

Table 5 : Treatment of fruit with NeemAzal-F:
(10 neonate larvae x 10 fruit)/treatment.

	Control	17 ppm	33 ppm	50 ppm
larvae fallen down (%)	21	13,7	17,5	23,8
total survival T = 7 d (%)	43,8 a	25 b	22,5 b	20 b

Table 6 : Treatment of fruit with NeemAzal-F
(5 neonate larvae x 10 fruit)/treatment.

	Control	12,5 ppm		25 ppm	50 ppm	100 ppm
larvae fallen down (%)	12	14	12	14	24	
total survival T = 7 d (%)	40	30	32	20	20	

DISCUSSION - CONCLUSIONS

In our field experiment, NeemAzal-F was not effective against an infestation by Myzus persicae which was already quite strong at the beginning of the experiment.

The effect of NeemAzal-F on first instar larvae and on the fecundity of the emerging adults that we observed in laboratory, suggests that there is probably a stronger effect of NeemAzal-F on young individuals than on already established colonies of aphids. Further field experiments should be made at an earlier time (for instance when the first females are observed).

In the field experiment we could not observe a direct effect of NeemAzal-F on syrphid and ladybird oviposition and larvae and on the common earwig, but laboratory tests showed long-term effects on syrphid and on earwig larvae.

The captures of earwig larvae in trap bands suggested that there is a repellent effect of NeemAzal-F. This could not be confirmed by the laboratory tests. But there was an obvious antifeedant effect on the common earwig and a molt-disturbing effect on syrphid larvae. These long-term effects, which could probably lead to an increase of the infestation, require further research.

On the oriental peach moth, the efficacy of 50 % of NeemAzal-F is not sufficient to control this pest, which has only few predators. Further experiments should be made to test the effect of a treatment on the first generation on shoots.

All these experiments must be considered as preliminary tests for the oriantation of future research.

REFERENCES

- ACTA, INRA, 1984. - Les auxiliaires - "Ennemis naturels des ravageurs des cultures" ACTA ed. PARIS,

- LENFANT C., SAUPHANOR B., 1992. - Des perce-oreilles dans les vergers. Qu'en faire? - Phytoma, in press.

- LYON J.P., GÖLDIN DE TIEFENAU P., 1974 - "Les Syrphes prédateurs de pucerons" - "Les organismes auxiliaires en verger de pommiers" - OILB-SROP; 163-170.

Erste Tastversuche mit NeemAzal-F (5 % Azadirachtin) gegen die Holunderblattlaus (Aphis sambuci) und hierbei beobachtete Nebenwirkungen auf Nützlinge (Marienkäfer, Schwebfliegen, Weichkäfer)

(First tentative experiments with NeemAzal-F (5 % Azadirachtin) against the elder bush aphid (Aphis sambuci) and thereby observed side-effects on benefitial insects (i.e. lady birds, hover-flys and Cantharid beetles)

R. BUSCH und H. TEUTSCH, Bezirkspflanzenschutzamt Koblenz, 5401 Emmelshausen

Unter dem Aspekt immer mehr sich verschärfender ökonomischer Zwänge sieht sich die landwirtschaftliche Praxis zunehmend mehr nach Produktionsalternativen mit höherer wirtschaftlicher Attraktivität um. Als eine der möglichen Alternativkulturen wurde in den letzten Jahren der Anbau von Holunder (Sambucus nigra) diskutiert. Seine Früchte finden in der Fruchtsaftindustrie (u.a. im Reformkostbereich) ebenso Verwendung wie zur Herstellung natürlicher Farbstoffe.

Investitionen in Form der Aufpflanzung entsprechender Anlagen sind jedoch nur dann verantwortbar, wenn der Absatz der Ernte durch Anbauverträge gesichert ist. Verständlicherweise sind gerade auf diesem Sektor Beschränkungen für den chemischen Pflanzenschutz häufig Bestandteil derartiger Abnahmeverträge. Daher erschien uns die Prüfung des Neem-Produkts in dieser Kultur besonders interessant und praxisrelevant, zumal die zunehmende Nachfrage nach alternativer Pflanzenschutzberatung in neuen Kulturen bisher nur unzureichend befriedigt werden kann.

Die Holunderblattlaus siedelt sich bevorzugt an den jungen Trieben in deren Spitzenbereich wie auch im Bereich der Blütendoldenverzweigungen an. Durch ihre Saugtätigkeit wird einerseits der Triebzuwachs gehemmt und damit der Blüten- und Fruchtansatz für die Tragruten des Folgejahres reduziert, andererseits der Assimilattransport in die Beeren vermindert und das Erntegut sowohl direkt als auch durch Ansiedlung von Rußtaupilzen verschmutzt bzw. unansehnlich gemacht. Der Umfang der potentiellen Schäden ist bisher nicht klar quantifiziert. Die Bekämpfungswürdigkeit der Läuse steht jedoch weitgehend außer Zweifel.

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Versuchsanlage:

Versuchsort:	5401 Urmitz/Rhein (Mittelrheintal)
Kultur:	Holunder, einstämmig auf Unterlage veredelt, Sorte: Haschberg, 2. Standjahr
Anlagengröße:	0.25 ha
Parzellengröße:	2 Bäume
Wiederholungen:	4
Behandlung:	Rückenspritze, 5 bar, tropfnaß
Behandlungstermin:	24.05.91 - kurz vor Beginn der Blüte

Versuchsvarianten:

Vgl. 1	=	Kontrolle (starker Vorbefall: ca. 10 ausgedehnte Kolonien pro Baum)
Vgl. 2	=	NeemAzal-F 0.01 %
Vgl. 3	=	" 0.05 %
Vgl. 4	=	" 0.1 %
Vgl. 5	=	Zweite Kontrolle (geringer Vorbefall, beginnende Blattlausbesiedlung)
Vgl. 6	=	NeemAzal-F 0.1 %

Versuchsergebnis:

Anzahl lebender Blattläuse je 5 Befallsstellen (1 Befallsstelle in Unbehandelt ≈ 220 Läuse ausgezählt)

Vgl.		24.05 (Vorbefall)	07.06. (14 Tage)	WG nach Abbott (%)
1 Kontrolle I		1.100	900	18 (Aktivität der Nützlinge)
2 NeemAzal-F	0.01	1.100	900	18
3 "	0.05	1.100	850	23
4 "	0.1	1.100	750	32
5 Kontrolle II		150	150	-
6 NeemAzal-F	0.1	150	50	67

Diskussion

Neben der Holunderblattlaus wurden am Behandlungstag in den Versuchsgliedern 1 - 4 je 5 Befallsstellen 10 Marienkäfer (Coccinella septempunctata), 3 Weichkäfer (Cantharis sp.) und 1 - 3 Schwebefliegenlarven (Syrphidae) ausgezählt.

Innerhalb einer Stunde nach der Behandlung lagen die Nützlinge samt und sonders unter den Bäumen auf dem Boden. Die Wirkung von NeemAzal kam dem Betrachter wie eine Schocktherapie vor. Bereits 3 Tage später hatten sie die Holunderbüsche jedoch wieder besiedelt und gingen fleißig ihrer Arbeit nach. Die kurzfristige negative Beeinträchtigung blieb ohne dauerhafte Auswirkung.

In den Anwendungskonzentrationen von 0.01 und 0.05 % (Vgl. 2 und 3) blieb NeemAzal-F ohne jede erkennbare Wirkung auf die Läuse. Nur bei 0.1%iger Anwendung konnte bei starkem Befall an Läusen eine schwache Zusatzwirkung durch die Behandlung bonitiert werden. Die ermittelten Wirkungsgrade gingen vorwiegend auf die Aktivität der Nützlinge zurück (18 %).

Ein besserer Erfolg wurde mit Versuchsglied 6 (Behandlung mit 0.1 % NeemAzal in die beginnende Kolonienbildung) erreicht. Bei einem Vorbefall von 150 Tieren an 5 Befallsstellen konnten sich unter ebenfalls tatkräftiger Mithilfe der zahlreich vorhandenen Nützlinge kaum echte Kolonien bilden.

Auf der vorhandenen Holunderkulturfläche war der Befall der einzelnen Bäumchen sehr unterschiedlich. So wurden außerhalb des Versuchs auch solche Bäume getrennt behandelt und entsprechend markiert, die am Behandlungstag noch befallsfrei waren (Vorbefall: 0; 0.1 % NeemAzal). Diese Bäume blieben bis zum Ende der Vegetation völlig frei von Läusen.

Diese ersten Ergebnisse sind ermutigend. Für eine fundierte Aussage sind jedoch weitere Versuche notwendig, eventuell mit Behandlungsfolgen ab beginnender Kolonienbildung. Ein entsprechender Versuch wurde in der laufenden Woche (25. Woche) angelegt.

Versuch über die systemische Wirkung von Neemextrakten auf Plutella xylostella

Martina Wendorf und Christian Schüler
Fachgebiet Methoden des Alternativen Landbaus
GHK, Nordbahnhofstr. 1a
3430 Witzenhausen

1. Material und Methoden

1.1. Eingesetzte Neemextrakte

Wasserextrakt aus Neemsamen

Samenkerne aus Tansania wurden vom Institut für Phytopathologie und Angewandte Zoologie Gießen zur Verfügung gestellt. Die Samenkerne wurden feingemahlen, im Verhältnis 1 : 20 mit dest. Wasser versetzt, 3 Std. mit einem Magnetrührer gerührt, dann 20 Std. stehen gelassen und anschließend zweimal mit einem Faltenfilter (Schleicher u. Schuell: 595 1/2, O 240 mm) filtriert. Bei diesem Extraktionsverfahren werden ca. 90% der Azadirachtine gelöst (Feuerhake, 1985). Der Kaltextrakt hatte nach Aufnahme eines HPLC einen Azadirachtingehalt von 0,104 mg/ml.

NeemAzal-F der Firma Trifolio-M GmbH

Im Vergleich zu dem hergestellten Kaltextrakt wurde ein angereicherter, bereits formulierter Neemextrakt der Firma Trifolio-M GmbH mit dem Produktnamen NeemAzal-F eingesetzt. Der Azadirachtingehalt beträgt 5 %.
Zusammensetzung von NeemAzal-F (500 ml):
Wirkstoffe: 50 g NeemAzal
Formulierung: 60 ml Brennspiritus, ca. 385 ml Tenside

1.2. Die Kohlmotte, Plutella xylostella

Zur Untersuchung der systemischen Wirkung der Neem-Extrakte wurde als Versuchstier Plutella xylostella eingesetzt. Die Kohlmotten entstammen einer Dauerzucht der biologischen Bundesanstalt Darmstadt. Die Zucht wurde im Labor des Fachbereichs ökologischer Landbau der GhK Witzenhausen zu Versuchszwecken fortgesetzt.

Während die Erstlarven mit ihrem Minierfraß nur geringen Schaden verursachen, können die weiteren Entwicklungsstadien, die erst zu Fensterfraß (Fraß an der

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Blattunterseite, wobei die obere Epidermis stehenbleibt) und dann zu Loch- bis Skelettierfraß übergehen, größere wirtschaftliche Schäden anrichten (Ertrags- und Qualitätsminderungen).

Der durch Plutella Larven verursachte Gesamtschaden ist von deren massenhaftem Auftreten abhängig. In den gemäßigten Breiten kann es besonders im August bei warmem, trockenem Wetter zu Massenauftreten kommen. In der Regel tritt ein temporärer Massenwechsel auf, der vor allem vom Klima und der Entwicklung von natürlichen Feinden der Kohlmotte bedingt wird. Die Kohlmotte ist ein weltweit verbreitetes Schadinsekt, das überall dort auftritt, wo Kohlanbau möglich ist. Allerdings ist ihre wirtschaftliche Bedeutung in warmen Klimazonen wesentlich höher. Dort hat sich bei Plutella nach intensiven Bekämpfungsmaßnahmen eine breitgefächerte Insektizidresistenz entwickelt, so daß eine Kontrolle des Schadinsekts auf diesem Wege kaum mehr möglich ist. (SCHMUTTERER und HOFFMANN 1983; FRANZ, KRIEG, 1982)

1.3. Markstammkohl

Als Testpflanze für den Ganzpflanzenversuch wurde Markstammkohl (Brassica oleracea, convar. aufphola, var. medullosa) verwendet, womit auch die Tiere der Dauerzucht gefüttert wurden. Markstammkohl gehört zur Gattung Brassica, die ihrerseits der Familie der Cruciferae angehört. Der Markstammkohl wurde in Multitopfplatten ausgesät und nach 3,5 Wochen in ein Erdgemisch, bestehend aus 1 Teil Sand, 1 Teil Kompost, 1 Teil Torf getopft. Das Gewicht des luftgetrockneten Substrates betrug 430 g pro Topf.

2. Versuchsdurchführung

2.1. Vorversuch zur Pflanzenverträglichkeit der Neemextrakte

Es wurde ein Vorversuch zur Pflanzenverträglichkeit der Neemextrakte durchgeführt, da über diesen Sachverhalt unterschiedliche Aussagen vorliegen, die von einer pflanzenstärkenden bis zu einer phytotoxischen Wirkung von Neemextraten berichten (STEETS 1976).

In diesem Versuch wurden je 4 Pflanzen mit verschieden hoch konzentrierten Lösungen aus NeemAzal-F gegossen.

1. Variante: 5 mg Azadirachtin pro Topf
   100 mg NeemAzal-F in 50 ml a.d.
2. Variante: 10 mg Azadirachtin pro Topf
   200 mg NeemAzal-F in 50 ml a.d.
3. Variante: Kontrolle, 50 ml a.d.

Die Varianten wurden danach 12 Tage lang beobachtet, es zeigten sich jedoch keine phytotoxischen Störungen bei den behandelten Markstammkohlpflanzen.

2.2. Versuch zur systemischen Wirkung der Neemextrakte

Die im Versuch eingesetzten Larven von Plutella xylostella entstammten Eiern, die im Flugkäfig in der Nacht von 28.6. zum 29.6. an Markstammkohlblättern abgelegt worden waren. Die Eier wurden im Thermostat aufbewahrt, in dem der gesamte Versuch unter folgenden Bedingungen stattfand:

Tagestemperatur: 20° C	Abs. Luftfeuchte: 60 %
Nachttemperatur: 17° C	Abs. Luftfeuchte: 85 %

Die systemische Wirkung wurde untersucht, indem die Markstammkohlpflanzen mit Lösungen der zwei Extrakte gegossen wurden und 4 Tage später Larven von Plutella xylostella auf einer Pflanze abgesetzt wurden, da nach Beobachtung anderer Autoren (J. Meisner; Venezia Melamed-Madjar; Shoshana Yarhom; aus K.R.S. Ascher, 1987) eine Pflanze einige Tage benötigt, um die Neeminhaltsstoffe aufzunehmen und in die Blätter zu transportieren. Zum Zeitpunkt der Versuchsdurchführung (ab 8.7.) waren die Larven 10 Tage alt und befanden sich bereits im 4. Larvenstadium.

Es gab 3 Varianten:

Variante 1: Kontrolle, giessen mit 50 ml a.d.

Variante 2: Giessen mit dem Wasserextrakt, 47 ml Wasserextrakt mit a.d. auf 50 ml aufgefüllt,
5 mg Azadirachtin pro Topf;

Variante 3: Giessen mit der NeemAzal-Lösung
100 mg NeemAzal-F in 50 ml a.d.
5 mg Azadirachtin pro Topf

Es wurden pro Variante 6 Larven ausgesetzt und 6 Wiederholungen durchgeführt. Damit die sehr mobilen Tiere die Pflanzen nicht verlassen konnten, wurden diese in eine Plastiktüte gestellt. Dazu wurde in die Topferde ein Drahtgestell als Stütze über die Pflanze gesteckt und darüber die mit Luftlöchern versehene Plastiktüte gezogen und am Topf mit einem Gummiband befestigt.

Nach dem Aussetzen der Larven konnten diese 8 Tage ungestört fressen. Ab dem 16.7. erfolgte dann jeden 2. Tag eine Kontrolle der Entwicklung der Tiere. Zu diesem Zeitpunkt war die Entwicklung der L4 der Kontrolle bereits abgeschlossen und schon 4 Falter geschlüpft, während sich bei beiden anderen Varianten bereits eine deutliche Entwicklungsverzögerung abzeichnete bzw. ein Teil der Larven bereits getötet waren.

3. Ergebnisse

In der nachfolgenden Tabelle werden die Beobachtungen im Versuchsablauf anhand der Anzahl Puppen, toter Larven und geschlüpfter Falter der Versuchsvarianten dargestellt.

	Variante 1		Variante 2			Variante 3		
Datum	P	M_a	P	M_b	tL	P	M_b	tL
16.7.	32	4	5		3	12		14
18.7.	11	25	13		7	13	1	19
20.7.	4	32	15		9	10	4	20
22.7.	2	34	15		13	10	6	20
24.7.	2	34	18	1	17	9	7	20
26.7.	2	34	17	2	17	9	7	20
28.7.	2	34	15	4	17	9	7	20

P = Puppen
M = Motten
tL = tote Larven

Die Differenz zwischen den 36 ausgesetzten Larven und der Anzahl der P, M, und tL, entspricht der Anzahl der noch lebenden Larven.

Varianten mit gleichen Buchstaben unterscheiden sich nach Tuckey ($p = 0{,}05$) statistisch nicht signifikant (Grenzdifferenz = 1,01).

Variante 1: Die 2 Puppen haben sich nicht mehr zu einem Falter entwickelt; ein Falter war verkrüppelt. Da die geschädigten Falter nicht vermehrungs- und überlebensfähig sind, gehen diese nicht in die statistische Berechnung mit ein.

Variante 2: Alle 15 Puppen haben sich nicht mehr zu Faltern entwickelt. Zwei der geschlüpften Falter waren verkrüppelt.

Variante 3: Auch hier haben sich die Puppen nicht weiterentwickelt, von den Faltern war einer geschädigt.

Bei einem großen Teil der Puppen der Varianten 2 und 3 zeigten sich Deformationen; einige Larven hatten zwar einen Kokon gebildet, sich dann aber nicht weiterentwickelt, bei anderen war nur teilweise eine Verpuppung zu beobachten, z.B. war der Hinterleib verpuppt, während das Vorderteil des Tieres noch die ursprüngliche Larvenform behielt. Andere Larven, die zwar eine vollständige Puppe ausgebildet hatten, wirkten im Vergleich zu den Puppen der Variante 1 verkümmert, waren meist kleiner oder zeigten eine unnatürlich gekrümmte Form.

Gesamtmortalität am Ende des Versuchs:

	Variante 1	Variante 2	Variante 3
%	8,3	94,4	83,3

Auf den letzten Seiten folgt eine graphische Darstellung des Versuchsverlaufs.

4. Diskussion

Eine systemische Wirkung von Neemextrakten konnte erstmals von GILL and LEWIS 1971 nachgewiesen werden. Die Erde junger Bohnenpflanzen wurde mit verschiedenen Neemsamenextrakten und Azadirachtin in verschiedenen Konzentrationen gegossen und dann der Wüstenheuschrecke (Schistocerca geregoria Fersk) zum Fraß in einen Käfig gestellt. Bis zu 25 Tagen nach der Behandlung konnte ein Schutz der behandelten Pflanzen nachgewiesen werden, die kaum Fraßschäden aufwiesen, während die Kontrollpflanzen kahlgefressen wurden.

STEETS konnte 1976 diese Beobachtung bei Versuchen mit Epilachna varivestis (Mexikanischer Bohnenkäfer) an Bohnenpflanzen bestätigen. Allerdings zeigten hier die Bohnenpflanzen phytotoxische Symptome.

Danach gab es noch wenige weitere Untersuchungen zur systemischen Wirkung von Neempräparaten.

Ein Versuch mit Liriomyza trifolii zeigte, daß die vollständige Aufnahme der Neeminhaltsstoffe einige Tage benötigt; die besten Ergebnisse wurden erzielt, wenn die Schädlinge 5 Tage nach Behandlung ausgesetzt wurden. (MEISNER et al. aus SCHMUTTERER, ASCHER 1987).

Auch in einer anderen Untersuchung, bei der Neemsamenpulver und Wasserextrakt zur Erde junger Kohlpflanzen gegeben wurden, konnte ein guter Schutz der Pflanzen vor Fraßschäden von L4 - Larven von Pieris brassicae bis 15 Tage nach Behandlung erreicht werden. Gleichzeitig konnte eine Düngerwirkung bei den Kohlpflanzen beobachtet werden. (OSMAN and PORT 1990).

In diesem Versuch konnte eine hohe systemische Wirksamkeit von NeemAzal-F und dem Wasserextrakt unter Laborbedingungen gut nachgewiesen werden.

Während bei den Plutella - Larven der Kontrolle, die Verpuppung am 16.7. schon vollständig abgeschlossen war und bereits 4 Falter geschlüpft waren, sind bei den mit NeemAzal behandelten Pflanzen lediglich 3 Puppen und eine große Anzahl getöteter Larven zu verzeichnen. Der Wasserextrakt zeigt zwar eine langsamere Wirkung und es erreichen wesentlich mehr Larven das Puppenstadium, wovon 50 % lebensfähig sind, liegt aber in der Gesamtmortalität etwas höher als NeemAzal-F.

Auch in der Kontrolle entwickelten sich 2 Puppen nicht zum vollständigen Insekt und ein Falter war geschädigt. Das läßt sich vielleicht durch die hohe Belastung der Tiere durch die hohe Luftfeuchtigkeit innerhalb der Plastiktüte erklären, die trotz einer starken Lochung entstand.

Die systemische Wirkung der Neemprodukte birgt einen weiteren Vorteil dieses natürlich Pflanzenschutzpräparates, da dadurch auch nachwachsende Blätter und Knospen weiterhin vor dem Befall von Schädlingen geschützt werden.

Während die Wirksamkeit von Azadirachtin unter Freilandbedingungen nach 5 - 7 Tagen durch Regenfälle und UV-Strahlung stark abnimmt, hält die Wirkung systemisch eingesetzter Extrakte über einen längeren Zeitraum an.

5. LITERATUR

FRANZ, Jost M., Aloysius KRIEG 1982
Biologische Schädlingsbekämpfung, Verlag Paul Parey, Berlin u. Hamburg;

FEUERHAKE, Karl 1985
Untersuchungen zur Gewinnung und Formulierung von Sameninhaltsstoffen des Neembaumes (Azadirachta indica A. Juss) im Hinblick auf ihre Verwendung als Pflanzenschutzmittel in den Entwicklungsländern, Dissertation, FB Angewandte Biologie und Umweltsicherung, Justus-Liebig-Universität Gießen;

GILL and LEWIS 1971
Systemic Action of an Insect Feeding Deterent , Nature Vol. 232, August 6, 402-403

HOFFMANN, G.M. und H. SCHMUTTERER 1983
Parasitäre Krankheiten und Schädlinge an landwirtschaftlichen Kulturpflanzen, Stuttgart, Ulmer

MEISNER, J.V. MELAMED - MADJAR, S. YATHOM u. K. R. S. ASCHER 1987
The influence of neem on the European corn borer (Ostrinia nubialis) and the serpentine leafminer (Liriomyza trifolii), aus: SCHMUTTERER, H., K.R.S. ASCHER 1987, S. 461-479

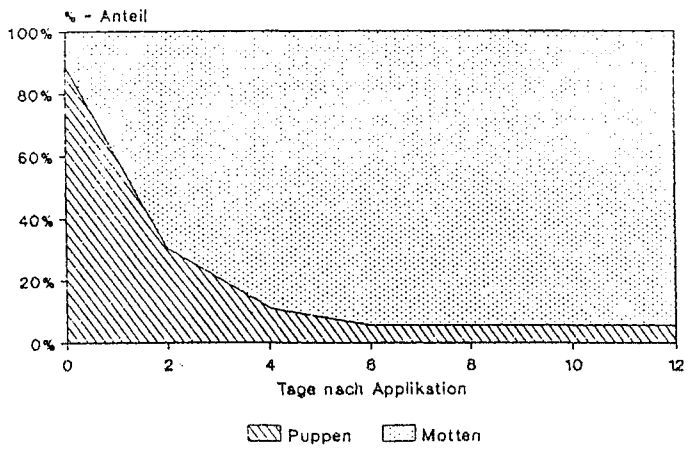
OSMAN, M.Z. and G.R. PORT 1990
Systemic action of neem seed substances against Picis brassicae, Entomol. exp. appl. 54

SCHMUTTERER, H., K.R.S. ASCHER 1987
Natural pesticides from the Neem Tree (Azadirachta indica A. Juss), Proceedings of the 3rd International Neem Conference, Schriftenreihe der GTZ Nr. 206, Eschborn

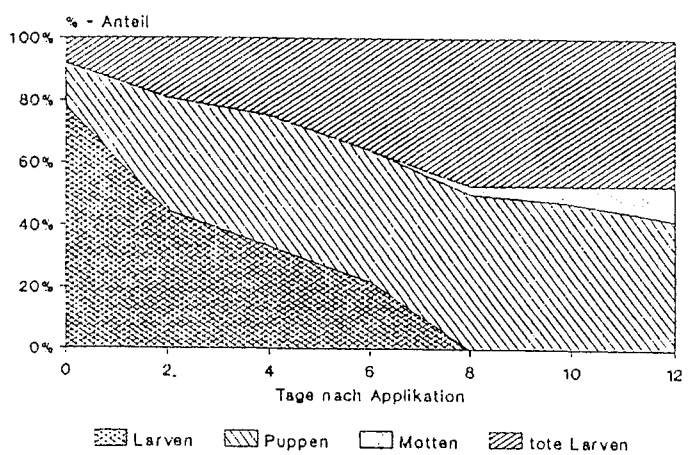
STEETS, Reinhold 1976
Zur Wirkung von Inhaltsstoffen aus Meliaceen und Anacardiaceen auf Coleopteren und Lepidopteren, Dissertation, FB Angewandte Biologie und Umweltsicherung, Justus-Liebig-Universität Giessen.

Graphische Darstellung des Versuchsverlaufs

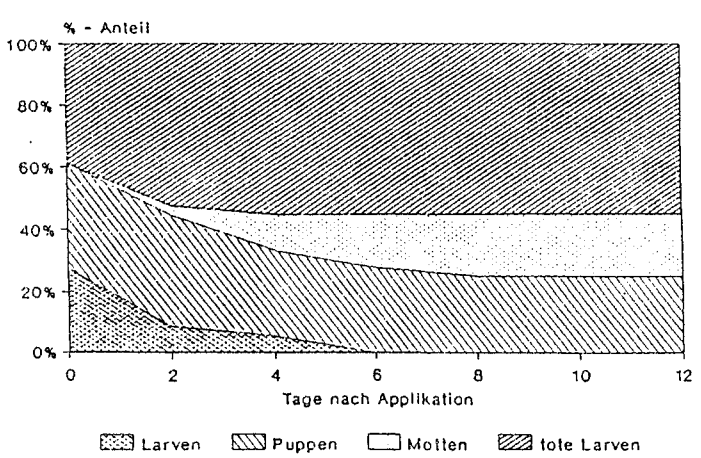
Variante 1



Variante 2



Variante 3



Entwicklungsverlauf der Larven in den Versuchsvarianten 1 (Kontrolle), 2 (Wasserextrakt) und 3 (NeemAzal-F).

Introduction of Neem as a Simple and Efficient Method of Pest-Control for the Nicaraguan Farmers

Bettina Mandellaub
c/o Hartmann
Südring 61
6500 Mainz-Bretzenheim

The Neem-Project in Nicaragua began officially in the year 1987.

It was subdivided into three parts: Part one was that Sub-Project, that had to introduce the method of using the Neem-water-extract, and especially to the cooperatives of the region, but also to small producers who were interested. Part two and three were responsable for the construction of a larger Neem-processing-plant and for extended Neem-plantations, respectively. These plantations could be even areas of the Nicaraguan government.

In the Sub-Project one, which we are speaking about today, we had to care of a district of almost 600 square kilometres, in the south pacific region of Nicaragua, which has maize as the main crop. We had the conception to plant 60 000 Neem-trees there, together with the farmers, and into their own territories. Those territories were possession of the cooperatives. Finally, after 5 years, we reached a number of 30 000 trees, but planted with the real intention to take care of them and to follow the line of our project. The other trees remained unplanted, either because of the low interest of some of the farmers, or because of their bad economical situation.

The trees were mostly planted in areas of 1 or 2 hectars or 1 to 2 "manzanas", which corresponds to 0,7 hectar, in a distance of 3 or 5 metres between each tree. After three years, the first plantations began to bloom and to bear fruits. Our first crop of Neem was in 1989, getting about 300 kilograms from 15 to 20 trees. In 1990 we harvested 1 850 kilograms from about 70 trees, and in 1991 we had 5762 kilograms from 300 trees. We are expecting 20 tons of fruit, when all the trees will take fruits. The trees are very different between each other with respect to the quality and quantity of fruits: Some of them bear 60 kilograms or more, and some only 3 or 5 kilograms. One kilogram of fruits in Nicaragua corresponds to 12 per cent of seeds with shell, so the last crop of 5 762 kg of fruits corresponds to 625 kg of seeds.

The hotter the climate of an area was, the more crop of fruits we got from the trees. From 1989 to 1991 we built a little processing-plant for the fruits, in the middle of the district, that could be reached easily by all the farmers (near the main-road to the

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

sea). This plant consisted of a peeling machine, of a hall, with some implements, as drying riddles and an electric mill for the seeds, and an open shadow-hall for the ripening of the green fruits. The locality of the plant was of a very hot, dry and windy climate, and so we almost had no attacks of Aspergillus flavus on the seeds. We hang the dry seeds up in jute-sacks, and left holes in the wall of the hall, so that the air could pass by all the day.

During the 5 years of our project, we made many instruction-days with the farmers, even as demonstration-experiments, in their own fields. The crops with which we mostly tested the Neem-water-extract, was: maize, cabbage and water-melon. In maize we found Spodoptera frugiperda, Heliothis zea, and sometimes Mosis latipes, in cabbage, Plutella xylostella and Estigmene, and in water-melon sometimes Spodoptera exigua.

Maize:

In the majority of our experiments in maize, we took an area in the middle of the field, of about 500 square metres. We counted the number of pest-insects. After the first 10 days of growth mostly there was an attack of 50 - 70 per cent of the plants (more than each second plant). After three applications of the water-extract in an average, we had a reduction of the pest to 10 % to 16 %.

Cabbage:

Here our areas were only of about 50 square metres. The main pest was Estigmene. We noticed a reduction from 40 % to 5 % of larvae, employing three times the extractat intervals of 10 to 12 days. The dose of the extract was of 50 - 60 g of ground seeds with shell in one litre of water. In the first two years we took 60 g, and then we saw that even 50 g was sufficient. The ground seeds were diped in a ton of water, remained there for 6 to 12 hours and then were applied on the field. Almost every time the reaction of the larvae was, first, that they became motionless, then they died after one or two days, changing their colour to brown. Later they fell down on the ground and died.

The most important thing in the application of the water-extract of Neem, is the manner <u>how</u> to apply it: one thing is the time of the day, in which it has to be applied: it must be the early morning, possibly before the sunrising, and the other thing is, <u>how</u> to spray it: the whole plant must be completely wet by the extract, and in maize, at least the center of the leaf-heart. It's always better, if the plants get covered by the liquid all over. It is not sufficient to spray the extract in the way of low-volume, because the plants never get covered completely.

A great problem for us was also the social component: The farmers hadn't ever heard before about Neem or other botanical methods of pest-control, and they sometimes reacted cutting the trees, because they didn't see any advantage in it. But after three years of experiments, more than 25% of them used Neem in a part of their crops, and this was a great success for us. They had to be accustomed, that the insects die in a slow way, and that they sometimes don't see an immediate effect.

The quantity of water they use on a manzana, varies between 60 to 150 litres; this means between 3 and 7.5 kg of ground seeds in one application. Totally we have about 15 kg of Neem-seeds to use on one manzana of a crop, in its vegetation-time.

At last we can say, that the use of Neem-seeds in pest-control is also an economical matter, because the production of 5 kg of Neem-powder (as an average value for an application), costs less than the same quantity of an imported chemical substance (8 $ against 10 - 15 $), not forgetting that Neem has no poisoning-effect for human beings.

FIRST EXPERIENCES WITH NEEM IN ITALY AND ITS POTENTIAL USES IN PLANT PROTECTION.

M.Chianella[a], L.Rovesti[b]

Summary

A summary of the experiences carried out in Italy by the SIAPA's Research Centre and the C.N.R. Pesticide Study Centre of Bologna is reported.
These studies have been conducted with formulations of neem seed oil and aqueous extracts, the chemical composition of which was unknown.
Neem showed insecticidal and fungicidal activity.
The oil formulations were generally phytotoxic, unlike the aqueous extracts.
In some trials a systemic or translaminar activity was observed.

Introduction

The neem tree, *Azadirachta indica*, of the botanical family of Meliaceae, has long been known in tropical countries for its numerous properties, amongst which also its pesticidal activity (Radwanski, 1977; Warthen, 1979). In the last decade neem has also attracted much interest in many developed countries, where the research in crop protection is aimed towards the development of the least toxic pesticides. In view of the positive results obtained so far in various research projects on natural pesticides neem is also presently considered in Italy as one of the most promising candidates.

Experimental applications in Italy

Already in the early 1980's SIAPA's Research Centre started the study of neem in Italy, followed from the second half of the '80s by the C.N.R. Pesticide Study Centre of Bologna.
SIAPA used several formulations of neem seed oil coming from different origins, but mainly from India, whereas the C.N.R. Pesticide Study Centre used an aqueous extract obtained by grinding and soaking neem seeds for approx. 24 hrs.
A summary of the results is reported in tabs. 1, 2 and 3.
The neem seed oil formulations used by SIAPA's Research Centre were all coded and contained a % volume/volume of a.i. as oil. In fact the exact chemical composition of the oil was unknown. Therefore, the dosage rates in the tables are reported as a.i., but they actually refer to pure neem seed oil.

a) SIAPA Research Centre, via Vittorio Veneto, 7, 40015 Galliera BO, Italy
b) CNR, Centro Studio Antiparassitari, via Filippo Re, 8, 40126 Bologna, Italy

Practice Oriented Results on Use and Production of Neem Ingredients; H. Kleeberg (ed.);

Tab.1 Summary of control trials on several pests

SPECIES	INSTAR	TYPE OF EXPOSURE	CROP or SUBSTRATE	FORMULATION	EFFECTIVENESS	BIBL.REF.
Blatta orientalis	neanids	contact	floor tiles	OSH/S 25 EC OHPP 20 EC	no effect (0.00005-2.5 ml a.i./m²)	SIAPA unpublished
Drosophila fasciata	adults	food	pabulum	WSDH/S 10.5 EC OSDH/S 25 EC OPTA/O 75 EC OHPP 20 EC	Effective only at more than 1000 ml a.i./100 lt.	SIAPA unpublished
	eggs & larvae				Very good at more than 500 ml a.i./100 lt.	
Musca domestica	adults	contact	paper cylinder	OSDH/S 25 EC	no effect (0.1-1000 ml ai/100 lt.)	SIAPA unpublished
	eggs & larvae	food	pabulum	ONCS/O 60 EC OPTA/O 76 EC	Medium (12 ml a.i./m²)	
Ephestia kuehniella	adults[c]	repellence	flour	OPTA/O 75 EC	Good (5 ml a.i./100 kg flour)	SIAPA unpublished
	eggs & larvae	food	flour			
Leucoptera malifoliella	eggs	contact	apple-leaves	aqueous extract	no effect[d] medium[e]	(2)
	larvae	leaf treatment			good	
	adults	repellence			no effect	
Mamestra brassicae	larvae	field trial	spinach	OSDH/O 70 EC	no effect[f] (300-1000 ml ai/100 lt.)	SIAPA unpublished
Trialeurodes vaporariorum	eggs	contact	bean leaves	OPTA/O 75 EC ONCS/O 60 EC OTCM 70 EC	good (phyto) (5000 ml ai/100 lt.) no effect (50-500 ml a.i) no effect(500-1000 ml ai)	SIAPA unpublished
	neanids	contact		OPTA/O 75 EC ONCS/O 60 EC OTCM 70 EC	good (phyto) (5000 ml ai/100 lt.) low at <500 ml a.i. no effect(500-1000 ml ai)	
	adults	repellence		OPTA/O 75 EC ONCS/O 60 EC OTCM 70 EC	very good (500 ml a.i./100 lt. no effect (50-500 ml a.i) no effect(500-1000 ml ai)	

continued overleaf

c) site chosen for oviposition.
d) sample received from Schmutterer
e) sample received from Hellpap
f) only 1 trial

SPECIES	INSTAR	TYPE OF EXPOSURE	CROP or SUBSTRATE	FORMULATION	EFFECTIVENESS	BIBL.REF.
Psylla pyri	neanids & nymphs	field trial	pear	ONCS/O 60 EC	low at 300-1000 ml a.i./100 lt.	SIAPA unpublished
Erithroneura spp. *Empoasea spp.*	neanids & nymphs	field trial	grape	NEEM DUST	no effect (50 kg dust/ha)	SIAPA unpublished
Leptinotarsa decemlineata	larvae	field trial	potato	OTCM 70 EC	no effect (700 ml a.i./100 lt.)	SIAPA unpublished
Epithrix hirtipennis	adults	field trial	tobacco	ONCS/O 60 EC	no effect	(8)
Panonychus ulmi	eggs	contact	bean leaf disks	OPTA/O 70 EC	good (300-500 ml a.i./100 lt.)	(4)
	mixed population	contact	bean leaves	OSDH/S 25 EC OSDH/O 70 EC OPTA/O 75 EC ONCS/O 60 EC OTCM 70 EC	good (100-500 ml a.i./100 lt.)	
Tetranychus urticae	mixed population	field trial	soybean	ONCS/O 60 EC	good (300-1000 ml a.i/100 lt.)	SIAPA unpublished

Tab.2 Summary of control trials on several fungal plant diseases.

SPECIES	TREATMENT	CROP or SUBSTRATE	FORMULATION	EFFECTIVENESS	BIBL.REF.
Sphaeroteca pannosa var.*rosae*	curative	rose	OSDH/O 70 EC	no effect (250-500 ml a.i./100 lt.)	SIAPA unpublished
Sphaeroteca fuliginea	preventive curative	vegetable marrow	aqueous extract OTCM 70 EC	good good (1260-2520 ml a.i/100 lt.	(3) SIAPA unpublished
Puccinia recondita var.*tritici*	contact	wheat	aqueous extract	low	(3)
Erysiphe graminis var.*tritici*	preventive	wheat	aqueous extract OSDH/O 70 EC OTCM 70 EC	good good/very low persistence (1000 ml a.i./100 lt.)	(3) SIAPA unpublished
	curative	wheat	aqueous extract	good	(3)
Erysiphe graminis var.*hordei*	preventive	barley	aqueous extract	good	(3)
	curative		aqueous extract OTCM 70 EC	good low (500-1500 ml a.i/100 lt.)	(3) SIAPA unpublished
Erysiphe cichoracearum	preventive	cucumber	OTCM 70 EC OSDH/O 70 EC	good (250-350 ml a.i./100 lt.)	SIAPA unpublished
	curative				
Phytophtora infestans	preventive	tomato	aqueous extract	no effect	(3)
Cercospora beticola	preventive	sugarbeet	aqueous extract	no effect	(3)
Septoria apiicola	preventive	celery	aqueous extract	no effect	(3)
Penicillium spp.		in vitro	OTCM 70 EC OSDH/O 70 EC	no effect (31-1000 ppm a.i.)	SIAPA unpublished
Alternaria brassicicola		in vitro	OTCM 70 EC OSDH/O 70 EC	no effect (31-1000 ppm a.i.)	SIAPA unpublished
Fusarium spp.		in vitro	OTCM 70 EC OSDH/O 70 EC	low (125-1000 ppm a.i.) no effect (31-1000 ppm a.i.)	SIAPA unpublished
Botrytis cinerea		in vitro	OTCM 70 EC	low (31-1000 ppm a.i.)	SIAPA unpublished
Pythium spp.		in vitro	OSDH/O 70 EC	no effect (31-1000 ppm a.i.)	SIAPA unpublished

Tab.3 Summary of selectivity trials on several crops and on entomopathogenic nematodes.

TARGET	SPECIES	STAGE	FORMULATION	SELECTIVITY	BIBL.REF.
Crops	Apple	leaves	aqueous extract	Selective	(2)
	Wheat		aqueous extract	Selective	(3)
			OPTA/O 75 EC OSDH/O 70 EC OTCM 70 EC	< 5000 ml a.i./100 lt. < 5000[g] ml a.i./100 lt. < 5000 ml a.i./100 lt.	SIAPA unpublished
	Barley		aqueous extract OTCM 70 EC	Selective	(3) SIAPA unpublished
	Soybean		OSDH/O 70 EC	Selective	SIAPA unpublished
	Tomato		aqueous extract	Selective	(3)
			OPTA/O 75 EC OSDH/O 70 EC	< 5000 ml a.i./100 lt. < 5000[g] ml a.i./100 lt.	SIAPA unpublished
	Cucumber		OPTA/O 70 EC OSDH/O 70 EC OTCM 70 EC	< 500 ml a.i./100 lt. < 5000 ml a.i./100 lt. < 1000 ml a.i./100 lt.	SIAPA unpublished
	Bean		OPTA/O 75 EC	< 5000 ml a.i./100 lt.	SIAPA unpublished
	Sugarbeet		aqueous extract	Selective	(3)
	Celery		aqueous extract	Selective	(3)
	Vegetable marrow		aqueous extract OTCM 70 EC	Selective < 1000 ml a.i./100 lt.	(3) SIAPA unpublished
	Eggplant		OPTA/O 75 EC OSDH/O 70 EC	< 5000 ml a.i./100 lt.	SIAPA unpublished
	Potato		OPTA/O 75 EC OSDH/O 70 EC	Selective	SIAPA unpublished
	Pepper		OPTA/O 75 EC OSDH/O 70 EC	< 5000 ml a.i./100 lt.	SIAPA unpublished
	Sinapis		OPTA/O 75 EC OSDH/O 70 EC	< 5000 ml a.i./100 lt.	SIAPA unpublished
Nematodes	*Steinernema* spp.	J_3	aqueous extract	lethal at high concentration	(1)
	Heterorhabditis spp.				

g) symptoms appeared at 7 DAT

Discussion

On the basis of all the results reported in tab.1, it appears that neem is active against most of the tested pests. Both the oil formulations and the aqueous extract appear to have mainly IGR or antifeedant activity. Only the highest rates showed insecticidal activity.
The acaricidal activity was very interesting (Chianella et al., 1990), but it is possible that it was due to the presence of oil. The compound resulted active both as an ovicide and as an adulticide. In the ovicide activity tests it was observed that some neanids hatched from the eggs, but when they died their bodies were white and completely transparent. This could be mainly due to an antifeedant activity rather than to a direct activity on the new-born mites. Furthermore, in the trials on the adults it was observed that by treating half of the leaves the adults moved to the untreated part, thus showing repellent or antifeedant activity.

This repellent activity was also observed for *Ephestia kuehniella* adults, when they were introduced in a cage with two flour containers (treated and untreated). They laid their eggs only on the untreated flour.

Of all the pests tested, the leafminer *Leucoptera malifoliella* appeared to be most susceptible to neem. Although the effect on eggs or adults was very poor or completely ineffective, larvae were very susceptible, particularly when treated at early stages. In particular, a rate as low as 1.25 g kernels/litre applied on eggs reduced the pupation of the mature larvae by more than 80%, whilst no pupation occurred at 5 g/litre (whether applied on eggs or L1 larvae). Almost complete control was also achieved by spraying the leaves when the mining larvae were in the 2nd-3rd instar. However, in this case the delayed action of neem caused some damage.

Besides the translaminar activity on the leaves, the aqueous extract also showed systemic activity whether applied as a soil drench or when sprayed on the lower leaves of infested twigs. Translaminar activity was also observed in trials with *Panonychus ulmi*, when bean leaves were treated only on the upper surface but the control was obtained against the mites feeding on the lower leaf surface.

As regards the phytopathogenic fungi, interesting activity of both neem oils and aqueous extract was observed against powdery mildew on different host plants. In fact, the extract gave as good control as sulphur, when applied preventively or curatively. As for the seed oil formulations, the activity was good, but due to their high phytotoxicity it was not possible to determine the right dosage rate. Of the other fungi tested, only wheat rust (in vivo tests) appeared to be susceptible.

The oil formulations resulted very phytotoxic at high dosage rates on several crops. The symptoms were mainly the detachment of the epidermis, leaf burning and blockage of the vegetative growth. The more susceptible crops were the cucurbits.

In view of the previous nematode management reports in the literature, the negative effect on the entomopathogenic nematodes was expected. *Heterorhabditis* spp. tended to appear more susceptible than *Steinernema* spp. It should be pointed out that the nematodes were negatively affected

only by the highest concentrations tested. Nevertheless, caution will be necessary when applying these nematodes in control programmes where neem products are also used.

Conclusions : potential uses of neem in Italy

The present data clearly indicate that neem could be used in plant protection against several pests and plant diseases which are economically important in Italian agriculture. This double activity is worth stressing, since neem products have always been regarded only (or mainly) as insecticides. In fact, powdery mildews constitute one of the main problems in warm climates, and the good activity of neem against these fungi (and possibly others) may mean remarkable savings in costs and labour, as specific fungicidal treatments would not be needed.

The systemic and translaminar activity of neem is another important feature (uncommon amongst biological products, whether botanical or microbiological) which undoubtedly plays in favour of neem and may make it competitive even with the most recent pesticidal molecules.

However, the Italian legislation, similarly to that of other EEC countries, requires the registration of all pesticides, whether chemical, botanical or microbiological, and therefore, even neem products will have to undergo the same procedure. This means that detailed properties of the product must be submitted before any registration is granted. This rules out the use of crude preparations like those so far tested in Italy and reported in this review.

The commercial development of neem in Italy will therefore, depend upon the establishment of a product with a defined and standardized composition, supported by a complete package of toxicological and activity data.

References

1) Rovesti L., Deseö K.V. (1990). "Effect of neem kernel extract on Steinernematid and Heterorhabditid nematodes". Nematologica (35), 493-496

2) Rovesti L., Deseö K.V. (1991). "Effectiveness of neem seed kernel extract against Leucoptera malifoliella Costa (Lep.Lyonetiidae)". J.Appl.Ent. 111 231-236

3) Rovesti L., Di Marco S., Pancaldi D. (1991). "Effect of neem kernel extract on some phytopathogenic fungi under greenhouse conditions". Zeitschrift für Pflanzen Krankheiten und Pflanzenschutz, in press

4) Chianella M., Albertini A., Mallegni C., Trombetti G. (1990). "Studio sull'impiego di estratti di neem".

5) Rembold H. "Isomeric Azadirachtins and their mode of action". Focus on Phytochemical Pesticdes, Vol. 1: The Neem Tree, p. 47-67.

6) Radwanski S. (1977). "Neem Tree 3: Further uses and potential uses". World Crops Invest., July/August: 167-168.

7) Warthen J.D. (1979). ***Azadirachta indica***: a source of insect feeding inhibitors and growth regulators". U.S. Dept. Agric. Rev. Man., Northeast, Ser.4.

8) Balbiani A., Sannino L., Piro F. "Prove di controllo dell'Altica del tabacco (*Epithrix hirtipennis* Melsh.) mediante trattamenti liquidi ad applicazione fogliare". Informatore Agrario, in press

The effect of Neem oil, Neem Azal-F and Neem oil enriched on the mortality and fitness of adult Schistocerca gregaria (Forskal)

Report on investigations carried out in the Southern Tamesna-Desert (Republic of Niger)

H. Wilps[1], O. Nasseh[2] and S. Krall[2]

1 Forstzoologisches Institut
7801 Stegen-Wittental, FRG

2 Gesellschaft für Technische Zusammenarbeit (GTZ)
P.O. Box 51 80
6236 Eschborn 1, FRG

Abstract:

Various neem products were tested against resting and flying **S. gregaria**. Two of the products namly neem Azal-F and the unclarified neem oil were obtained from the Company Trifolio whereas neem oil enriched and pure neem oil were a gift of Prof. Schmutterer (University of Giessen). The treatment during flight activity caused for all products applied an increase of the mortality rate, except the pure neem oil of Giessen, up to 70 and 90 % respectively. The same products, however, sprayed on resting locusts did not show any remarkable mortality. But this treatment reduced the fitness of the locusts in terms of their flight performance as well as their adipokinetic potency. In consequence of this it is to expect that neem treated locusts will not be able to cover long distances. That means the lipid mobilizing system necessary to provide the flight muscles with "fuel" (lipids) is disturbed severely.

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Key words:

Schistocerca gregaria, Neem oil, Azal-F, flight application, flight performance, adipokinetic response

Introduction:

As past experience has shown, it will never be possible to completely prevent the formation of locust swarms, for a host of different reasons. This does not mean, however, that the control measures that are therefore required must necessarily aim at killing all of the insects or at least as many as possible; rather, the goal in each case will depend on the location. If the swarms have allready reached farmed areas, then high mortality rates are a must. That is why the mortality rates were determined first in the trials, although the aim was not primarily to induce high mortality rates no matter what the cost; the quantities of products used were based on practical considerations.

Therefore the following objectives were pursued:

1. Determination of induced mortality rates as a function of different products and application methods.

2. Quantification of the fitness of the surviving individuals in terms of flight performance and adipokinetic response, i.e. the extent to which these insects were physiologically still able to supply their flight muscles with sufficient quantities of lipids ("fuel") for extended flight.

In the following experiments, the mortality rates of adults after application of different neem products during flight and while resting on the ground were ascertained. The reasons for applying these substances to flying insects with the aim to control flying swarms, were as follows: treatment of flying swarms has practical advantages because flying

insects can be treated at any time during the day but resting swarms are only accessible during the hours of the evening and the early morning, when the duration of light is very short and visibility is typically very poor. Moreover, since the swarms fly several hundred metres above the ground during intensive migratory phases, the clouds of sprayed insecticide are rapidly dispersed, i.e. affected areas become less contaminated (Symmons, 1992, unpublished data). The locust themselves, however, absorb greater quantities of the active substances because they fly directly through the sprayed clouds and their entire body is exposed to the insecticides for long periods of time.

As regards the insects, furthermore, the following considerations are important:

According to results performed by Weiss-Fogh (1952), the oxygen uptake of flying insects increase by a factor of 50 to 100, associated with a similar intensification of metabolic rate. As a result, substances applied using ULV techniques can be directly absorbed owing to the increase respiratory activity, being subsequently distributed throughout the insect body because of the simultaneously high metabolic rate.

In-depth fitness studies of adult **S. gregaria** illuminated the adipokinetic response of treated an untreated locusts following injection with synthetic adipokinetic hormone (AKH). Because of the limited amount of time available, it was only possible to conduct isolated tests for determining the flight performance of these insects. In their entirety, these trials constituted a continuation of the experiments performed in Abangharit (Republic of Niger) in 1990 (Nasseh and Freres, 1990; Freres and Nasseh, 1990; Wilps and Nasseh, 1990).

Materials and Methods:

Adult locusts **S. gregaria** were collected between 6.30 and 11 a.m. in a Shouwia field 30 km east of Anou Mekkerene in the foothills of the Air

Mountains, and kept in the field cages for about 72 hours prior to beginning the experiments. During the trials, which lasted for 3 months, the days and nights were of equal length (12 hours each), and the daytime and nighttime temperatures were 40° ± 5°C and 25° ± 5°, respectively. The relative humidity was 10 - 15 % during the daytime, reaching maximum levels of 25 - 30 % at night. The insects were fed each day with fresh Schouwia thebaica plants.

Products used and quantities applied:

All of the products and control substanceds were applied using a hand sprayer (Micro Ulva).

Neem products:

In the studies described here, various neem products from different sources were used in varying concentrations.

a) Enriched neem oil, azadirachtin content: 0,2 % (60 g AZT extract + 439 ml propyl alcohol + 360 ml Span 85 per 3000 ml of neem oil).

b) Pure neem oil, origin of seeds: Republic of Niger; azadirachtin content: 0.01 %.

Both of these products were applied at a rate of 10 liters per hectare, and were supplied to us by Prof. Schmutterer of the University of Giessen.

c) Azal-F: 80 % filler (blank) + 20 % neem seed extracts; containing 5 % azadirachtin.

d) Pure neem oil: 0 04 % azadirachtin and 0.04 % 3-tigloyl-azadirachtol + approx. 10 % suspended matter.

These products were produced by the Trifolio-M Company (Lahnau, Germany) and applied at rates of 0.5 and 1.0 l/ha.

Controls:

The following controls were used in all trials:

a) Untreated insects

b) Insects that had been treated with the pure formulation substances and/or additives (vegetable oil, propyl alcohol + Span 85).

"Ground application":

The insects were first immobilized by cooling them to about 4°C by keeping them in a refrigerator for 20 minutes, then placed in an arena measuring 16 m^2 in size and fenced by plastic sheet (1.5 m high) and sprayed with various products (Micro Ulva, 1 and 10 l/ha).

"Flight application":

10 insects at a time flying on a flight mill were treated with the indicated substances using ULV techniques. The high of application was 60 cm. The application rates were calculated based on the sprayed volumes per unit of time and the size of the contaminated ground surface (determined by laying out oil-sensitive paper); they were either 2 or 10 l/ha. Each of the tested populations comprised 20 to 50 individuals, i.e. these applications had to be repeated 2 to 5 times per population. After spraying, the insects flight was continued for another 3 minutes.

Injection and incubation trails with synthetic adipokinetic hormone:

These trials were performed using untreated control insects and insects sprayed with various extracts using different methods. Prior to beginning the trials, the individual insects were allowed to rest by themselves for

1.5 to 2 hours. After removing 1 µ l of hemolymph (from the joint membrane of a hind leg), they were injected with 10 pmol of sAKHI dissolved in 10 µ l of distilled water, using a Hamilton syringe. The injection was performed ventrally through the intersegmental membrane between the 1st and 2nd abdominal segments. After an incubation period of 60 minutes, another 1 µ l of hemolymph was taken. The hemolymph samples were blown into 100 µ l of concentrated H_2SO_4; their lipid content was photometrically determined after hydrolysis using the vanillin reaction (Zöllner and Kirsch, 1962).

Flight trials:

An alligator clip that had been filed down to the shape of the pronotum was affixed to the insects, and they were then suspended in the flight mill in such a way that they could deflect out horizontally as a function of their flying speed. This approach permitted testing without causing any injury, so that the same insects could be tested more than once. The total number of rounds flown by 10 insects was captured by a programmable counting device that was operated by an injection coil mounted in the flight mill axis. The outdoor temperatures during all flight trials were between 30°C and 34°C.

Results and Discussion
Flight and ground application

The two products made by Trifolio and the enriched oil from Giessen were applied to both resting and flying locusts. The respective mortality rates thus induced are shown in figure 1 and 2 respectively.

Application of Trifolio neem products to resting insects resulted in 20 - 25 % mortality, while the enriched neem oil from Giessen only induced slightly higher mortality rates than those exhibited by the untreated controls.
The mortality rates following application of the same products to flying locusts are shown in the next figure.

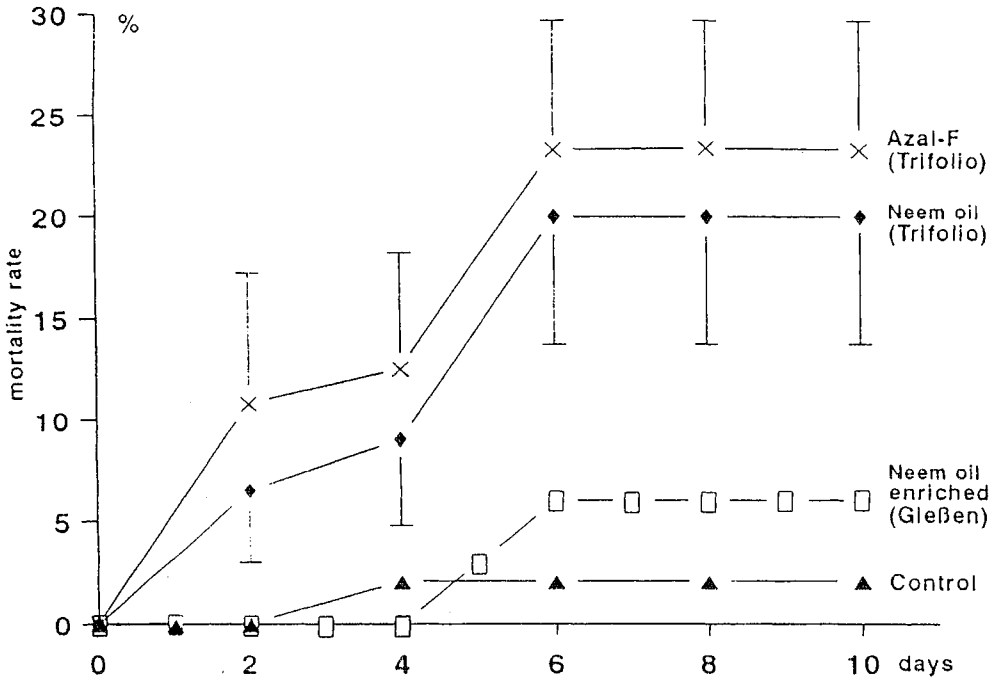


Fig. 1

Mortality rates of S. gregaria following application on the ground. The data are the average values ± SD from 4 trials with 30 insects each. The values without SD were obtained from a trial with 120 insects.

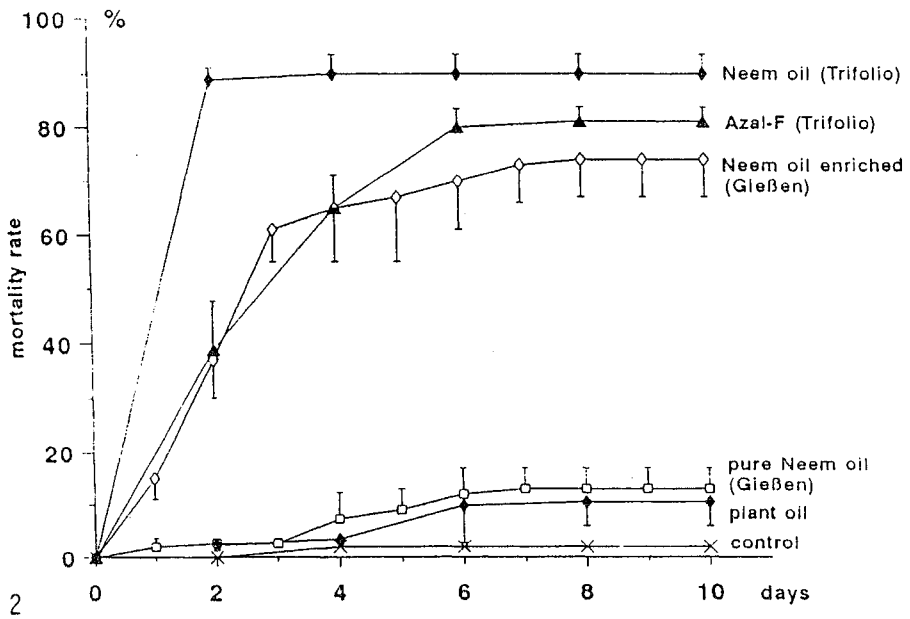


Fig. 2

Mortality rates of S. gregaria after flight application. The data represent the average values ± SD of 3 to 4 trials each with 30 to 48 locusts/trials. Because of the limited capacity of the flight mill, the application process had to be repeated 2 to 7 times for each population.

The neem oil from the Trifolio Company, which had an azadirachtin content of 0.04 % and an equally high concentration of 3-tigloyl-azadirachtol, inflicted nearly 90 % mortality within 2 days. The product Azal-F made by the same company and the enriched neem oil from Giessen were somewhat less effective and slower to act. They resulted in mortality rates between 70 % and 80 % over a period of 6 days.

Compared to the previosly mentioned products, neither the pure neem oil from Giessen (azadirachtin content: 0.007 %) nor the pure vegetable oil applied as a control led to a significant rise in mortality compared to untreated insects.

Studies of fitness reduction:

1) Loss of flight obility

In extended experiments the flight performance of ground and flight treated locusts as well as of untreated and vegeatable oil applied locusts was tested (fig. 3). Untreated and vegetable oil treated **S. gregaria** covered a distance of 5.600 m per hour nearly. However, the flight performance of the neem treated locusts is reduced drastically. Whereas the ground applicated locusts flew nearly the half of that distance covered by the controls, the flight treated **S. gregaria** reached only one third of the controls' achievement. Furtheron, the former were not able for a sustained flight activity taking more than 30 minutes time. After this time it was completely impossible to force the locusts to any further flight activity even by giving intensive mechanical and optical stimuli.

2) Decrease of adipokinetic response

S. gregaria, like nearly all other insects capable of flying long distances, mobilizes lipids stored in the fat body to meet the energy requirements for sustained flight. These lipids are transported in the

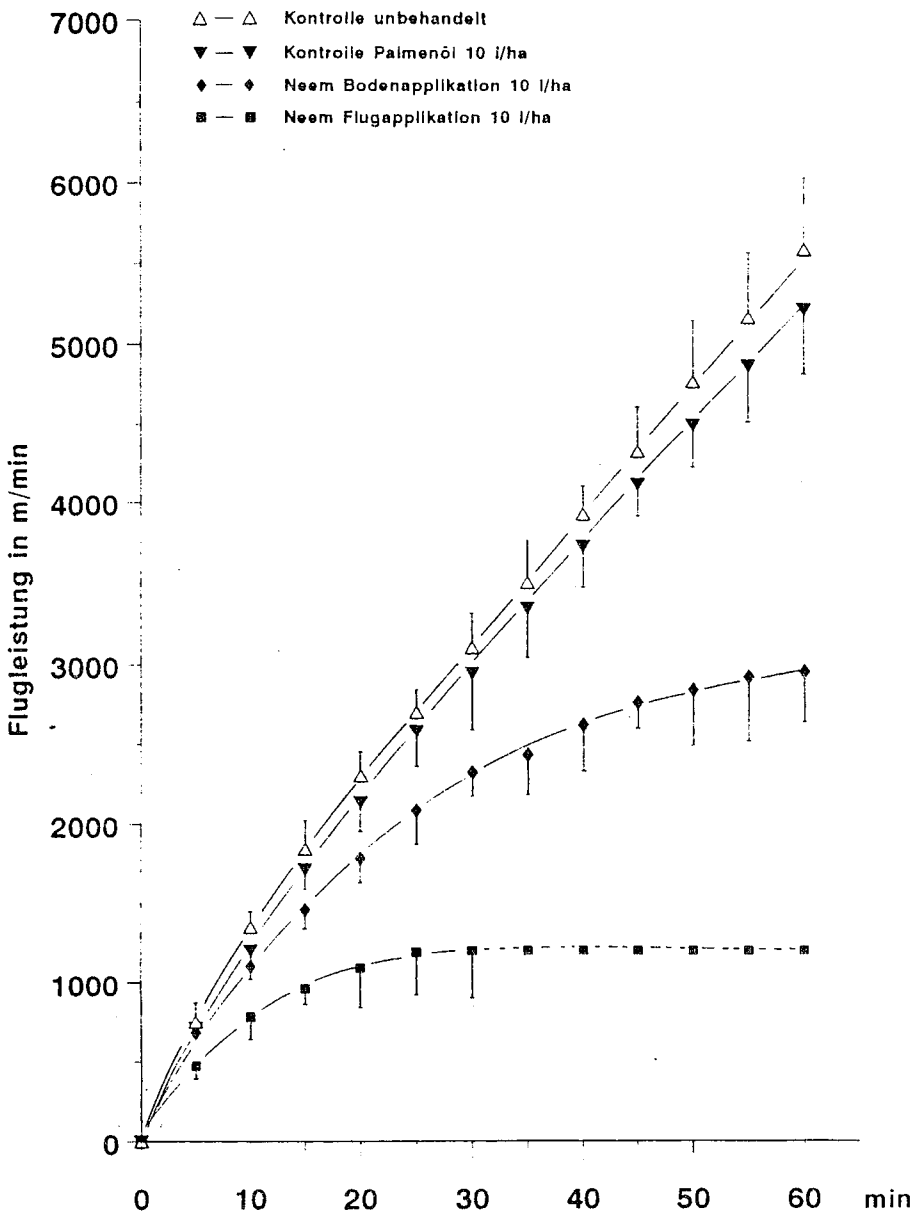


Fig. 3
Flight performance of untreated and palm- and neem oil (ground-flight) treated S. gregaria during one hour (c.f. text). The values are the average ± SD of 3 to 6 experiments for each population.

hemolymph to the flight muscles, where they are completely oxidized to CO_2 and H_2O. If it is possible to intervene and reduce this metabolic activity, then this has two consequences for **S. gregaria**. One is that the insect then lacks the fuel it needs for flight activity, and the other is that its water regime is disturbed. In the extremely arid regions inhabited by these insects, the water from oxidation of lipids and other foodstuffs is needed by them to regulate processes at the cellular level.

Lipid release is regulated by adipokinetic hormones (AKHs), which are synthesized by and stored in the corpora cardiaca. At the onset of flight activity, AKHs are released and stimulate the lipase system of the fat body.
In most of the laboratory investigations conducted so far, both extended flight activity and the injection of synthetic adipokinetic hormone have led to increased lipid concentrations in the hemolymph. These concentrations can be utilized as a yardstick for measuring the extent to which diminished flight performance is caused by inhibition or disturbance of the energy-supplying metabolic processes. By injecting synthetic adipokinetic hormone, it is possible to tentatively determine whether reductions in the energy-supplying metabolic processes. By injecting synthetic adipokinetic hormone, it is possible to tentatively determine whether reductions in the energy-supplying metabolism are induced by hormonal disturbances or lowered lipase activity in the fat body.

Fig. 4 shows the changes measured in the lipid content of the hemolymph of untreated control locusts after flight activity and AKH injection. Injection of AKH caused an increase in lipid concentration of nearly 30 mg/ml in both tested groups. A comparable increase was also exhibited by the laboratory insects in Freiburg after 60 minutes of flight. The results obtained with field locusts in Agadez are different; these had a declining lipid concentration after 30 and 60 minutes of flight. In other words, after beginning flight activity the lipids contained in the hemolymph are first used up, and then subsequently supplemented by the reserves in the fat body. As the equally high values after 30 and 60 minutes show, the insects' organisms settle into a floating equilibrium. If the locusts are kept quiet for another 30 minutes and their hemolymph

values measured again, it emerges that their lipid content has climbed back to the original resting levels. Since all of the groups responded identically to injection of AKH, it can be concluded that a hyperlipemic response was present in each of the tested groups. The differences between the laboratory and field locusts after 30 and 60 minutes of flight suggest that the field insects start out by consuming other fuels, e.g. carbohydrates, after beginning flight, a phenomenon that is known from other insects that fly long distances. In this case it may be related to the locusts' phase.

Since this unexpected finding must first be studied further and verified, it was decided to dispense with altering the lipid content of treated insects by inducing flight. Their adipokinetic responses following injection of AKH as a function of their prior treatments are shown in Fig. 5.

The two first columns show the changes undergone by the hemolymph lipid concentration of untreated control insects after injection of H_2O and AKH. The application of neem products to resting locusts (ground application) lowers their adipokinetic response by about 50 %. Taking account of the standard deviation, it can be assumed that the individuals surviving flight application suffer nearly total loss of their adipokinetic potency. In other words, as potency has already been shown by the flight trials (Wilps et al., 1991 a, b and c), these locusts are no longer capable of sustained flight.

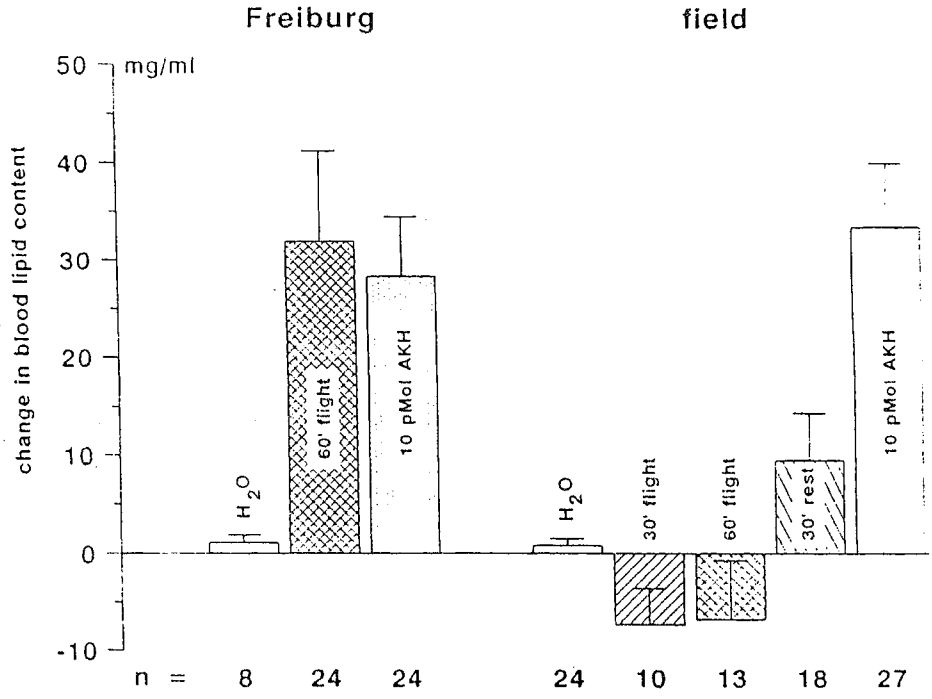


Fig. 4

Changes in hemolymph lipid content after flight activity and a subsequent 30 minute resting period vs injection of 10 p mol AKH. The data are the average of the injections indicated by n. H_2O control injections with water to ensure that the injections per se do not induce any changes in the lipid content. Freiburg: trials conducted with laboratory locusts in Freiburg. field: trials with field locusts in Agadez.

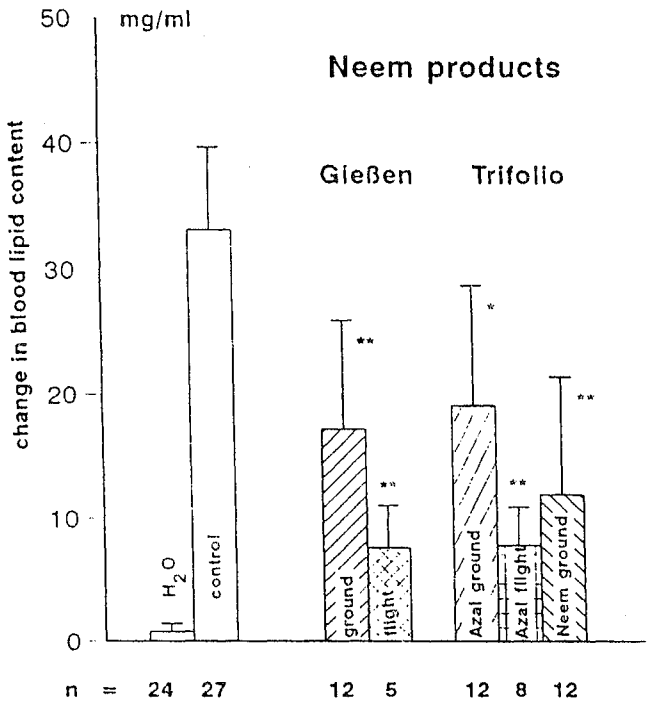


Fig. 5

Changes in lipid content following injection of S. gregaria with AKH 48 hours after treatment with neem products from Giessen or the Trifolio Company. In each case, the data are the average values ± SD from the number of trials given by n.

Ground: application to resting locusts on the ground. Flight: application to flying locusts. Control: untreated field insects. Control lab: untreated laboratory locusts. H_2O: control injections with water. Significant differences in comparison the controls are indicated by asterisks. Significance levels: * $p < 0.05$; ** $p < 0.001$.

Acknowledgments:

We are particularly indebted to Dr. U. Ch. Pantenius and Mr. Dipl.-Ing. J. Haag of the Niamey Crop Protection Project for their organizational assistance, as well as the helpers from Agadez and Anou Mekkerene. Gratitude is especially due to Mr. Al Hassan from the CNAA in Agadez, who led us to the locusts in the Air Mountains and whose eagerness to cooperate vastly facilitated our work in Agadez, and to Mr. Ihalen for his technical help with the flight applications, injections and photometric measurements. Last but not least, we extend our heartfelt thanks to Prof. H. Schmutterer, Dr. U. Hellpap and Dr. K. Ermel for having given us the neem products and sending us, precisely on time, large quantities of high-quality **S. gregaria** eggs. Also thanks to the universities of Constance, Oldenburg and Saarbrucken for supplying additional eggspods.

Literature:

Nasseh, O., and Freres, T. (1990)
Käfigfreilandversuche zur Bekämpfung der Larvenstadien und adulten Tieren von **Schistocerca gregaria** (Forskal) mit biologischen und chemischen Präparaten in der Republik Niger (Insenkat/Tamesna). In: GTZ-Schrift: Ergebnisse zur Bekämpfung der Wüstenheuschrecke **Schistocerca gregaria** in der Republik Niger.

Weis-Fogh, T. (1952)
Fat combustion and metabolic rate of flying locusts (**Schistocerca gregaria** Forskal).
Philos. Trans. Soc. London. B Biol. Sci. 237, 1- 36

Wilps, H., and Nasseh, O. (1990)
Untersuchungen über den Einfluß von Neemprodukten auf die Mortalität, Flugleistung und Regulation des energieliefernden Stoffwechsels adulter **Schistocerca gregaria**.
GTZ-report, Projekt-Nr. 89.2031.6-01.100, 1 - 19.

Wilps, H., Nasseh, O., and Krall, S. (1991a)
The effect of verious neem products on the fitness of adult Schistocerca gregaria.
In: Lomer, C., and Prior, C. (eds.) Biological control of locusts and grasshoppers. In: Biol. Contr. of locust and grasshoppers PP. 337 - 346

Wilps, H., Kirkilionis, E., and Muschenich K. (1991c)
The effects of neem oil and azadirachtin on mortality, flight activity, and energy metabolism of Schistocerca gregaria Forskal - A comparison between laboratory and field locusts. Comp. Biochem. Physiol. (in press).

Zöllner, N. and Kirsch, K. (1962)
Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophospho-vanillin Reaktion. Z. ges. exp. Med. 135, 545 - 561.

Efficacy of neem, *Azadirachta indica* A.JUSS, in controlling the laboratory reared and field captured larvae of *Schistocerca gregaria* (FORSK.) in field cage trials in the Republic of Niger

O.M.NASSEH *, H.WILPS * and S.KRALL *

Summary

Neem, *Azadirachta indica* A.JUSS, is an important evergreen tree which is planted along avenues and roads in Niger. Neem grows widely under divers ecological conditions in the country. In continuation of the GTZ Desert Locust Research Project, commercial and non-commercial preparations of neem oil were tested. The products were evaluated for their effect as botanical pesticides against the laboratory reared and field captured larvae of *Schistocerca gregaria* (FORSK.) on *Schouwia thebaica* (WEBB) plant. The application of neem oil was carried out with an ULV sprayer, Micro-Ulva, at a rate of 10 l, 1 l and 0.5 l/ha. All formulation of neem oil, were tested in large cages in the field, against 3rd to 5th instar laboratory-reared and field-captured larvae of the Desert Locust. Larval and adult survival, retard development, deformation and mortality served as the criteria of evaluation and were recorded at daily intervals after spraying. There was no statistical difference between the two commercial neem products applied at the rate of 1 l/ha. Both formulations were found to control significantly the larvae through either direct kill or interference with metamorphosis, leading to malformation and 100% death. The non-commercial neem products reached a maximum of 60% mortality.

* Deutsche Gesellschaft für Technische Zusammenarbeit/GTZ,

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Introduction

Chemical pesticides are still considered to be the most potent control technology for locusts and grasshoppers. Continous or heavy usage of some pesticides has created serious problems arising from contamination of the biosphere, toxicity to human beings, fish, predators and parasites. More than 800 pest species have developted resistance to chemical pesticides. The need for safer, more natural pesticides is now accepted in most countries without any serious contention. The Republic of Niger, with the help of developed countries, will take a decisive positive step in terms of developing, producing and using pest control remedies based on concepts of natural products. In this regard the integrated biological control of locusts and grasshoppers, a research project of the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), was stablished in 1990 with a base in Niger (KRALL and NASSEH 1990).

Attention has been devoted to the use of plant constituent that have an insecticidal effect as biocides. Since neem trees (*Azadirachta indica* A.JUSS) grow in Niger and it's well known that extracts of various parts of the neem tree, possess distinct insecticide and antifeeding effects against several species of insects, it was decided, therefore, to investigate the influence of neem oil on larvae of the Desert Locusts, *Schistocerca gregaria* (FORSK.).

The aim of using neem in Niger was motivated by major objectives: to minimize the contamination of the environment; to encourage use of locally available plant material; to indentify sources of botanical insecticides for commercial use; to substitute or supplement the activity of existing synthetic insecticides against

pests and to provide a focus for the development of information needed to effectively conduct IPM in controlling locusts and grasshoppers.

Materials and Methods

Enriched and pure oil of neem were obtained from "Institute for Phytopathology and Applied Zoology" University of Giessen, Germany and were applied at the rate of 10 l/ha.
The enriched oil from Giessen was coded AZT-VR-K. In 3 litre of oil, there are 60g AZT extracts + 439ml of propyl alcohol + 360ml span 85 with a total azadirachtin content of 0,2%. The pure neem oil was of 0.04% azadirachtin content. The following two neem products were supplied by the Trifolio-M Ltd, Germany and were applied in quantities of 0.5 l and 1 l per hectare. Neem Azal-F with 80% of blanc (formulation's product) + 20% Neem and it's derivates with a total azadirachtin contents of 5%. Unclarified neem oil with 0,04% of azadirachtin and 0,04% 3-Tigloyl azadirachtol + aprox. 10% suspended matter.
The laboratory locusts were raised from eggs in Agadez/Niger. The eggs were obtained from strains bred in Constance, Giessen, Oldenburg and Saarbruecken/Germany.
The field insects were collected in Äir/Niger. Both laboratory reared and field captured insects were kept outdoors in field cages meassuring 2x2x2 meters.
The daytime and night time temperatures were $45^{o} \pm 5^{o}$ C and $20^{o} \pm 5^{o}$ C, respectively, the relative humidity was 10 - 20%, and the days and nights were of equal lenth (12 hours each).
Larvae were treated early in the morning in an arena using a Micro-Ulva sprayer (Micron Sprayer Ltd, UK) with five batteries,

giving a disk speed of about 13000rpm. The trials were evaluated at daily intervals and were replicated four times, using 30 larvae of stages 3rd to 5th per replicate. 30 min. after the treatment they were released into the field cages.
Trial area, construction of the field cages and spraying technique are described in NASSEH et al 1991.
The main hostplant of *S. gregaria* in their recession areas in Niger, is the *Schouwia thebaica* (WEBB) (Brassicacae). Therefore five weeks old bushes of Schouwia were thinned to two plants of uniform growth per cage and served as a hostplant.

Results and Discussion

The objectives of these trials were: A- to ascertain whether neem products are capable, under desert conditions, of inducing mortality rates in *S. gregaria* larvae. B- to directly compare the effects of these products on laboratory-reared and field-captured larvae of *S. gregaria*.
Figure 1 shows the mortality rates of the larvae following application of the Trifolio-M product. Application of 1 liter of Azal-F per hectare killed 100% of the insects, both field and laboratory-reared larvae. However, the field larvae took 4 days longer to die than the laboratory-reared insects. The curves designated as blank indicate the results of the control trials. Blank "formulation's product", makes up 80% of Azal-F. The application of this substance by itself did not yield any significant increase in mortality rates, therefore similar trials were not performed with the field captured-larvae. Since the natural mortality rates of field and laboratory-reared insects differ considerably, on the 18th days, field larvae had a rate of

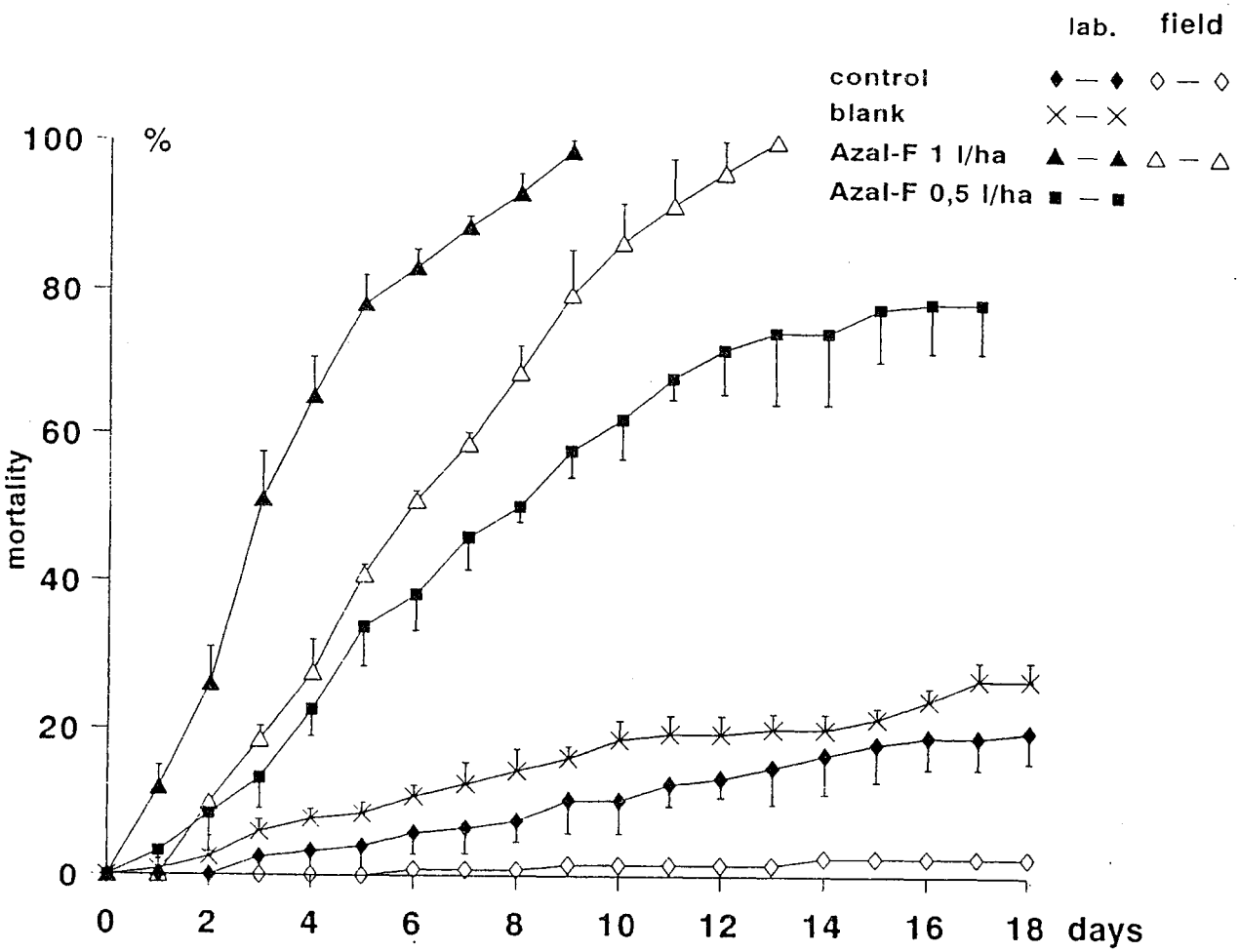


Fig. 1: Morality rates of laboratory-reared and field-collected larvae following application of Azal-F neem oil from the Trifolio-M company. Each of the individual values is the mean (+/- standard deviation) of 4 measurement values.
They represent the mortality rates of different populations resulting from the treatment performed on day 0 (cf. text).

less than 5% and the laboratory-reared insects roughly 20% - it is questionable whether laboratory findings can be transfered to field trials. These doubts are vindicated by the mortality rates in laboratory-reared insects following application of 0.5 l/ha of Azal-F. Until day 5, their curve is nearly identical with that of the field insects exposed to twice the concentration of Azal-F. The same results can also be observed after application of 1 l/ha of unclarified neem oil. Figure 2 indicates that this product also causes 100% mortality rates of the laboratory-reared larvae 4 days earlier than in the field-captured hoppers.

Figure 3 demonstrates the mortality rates of the larvae following application of the enriched neem oil prepared and formulated at the University of Giessen/Germany. The Fig. 3 shows that there was a difference between the respective maximum mortality rates: after 17 days, these were 53% for the field-captured hoppers and 60% for the laboratory-reared insects. However, with this enriched neem product, no time discrepancy was observed between the mortality rates of laboratory-reared and field captured hoppers.

Fig. 3 indicates further, that control population exposed to the formulation's product (Span 85 + propyl alcohol) also caused a slight, but not significant, increase in mortality.

Figure 4 shows the mortality of laboratory and field larvae after treatment with pure neem oil. The effects of time and the achieved mortality rates were very similar to those previously obtained with application of enriched neem oil, with the mortality rate of the laboratory-reared insects being significantly higher than that of the field larvae.

The neem products tested on *S. gregaria* larvae in the Tamesna Desert/Niger yield, from the viewpoint of effective pest control, satisfactory to 100% mortality (NASSEH et al., in preparation). In

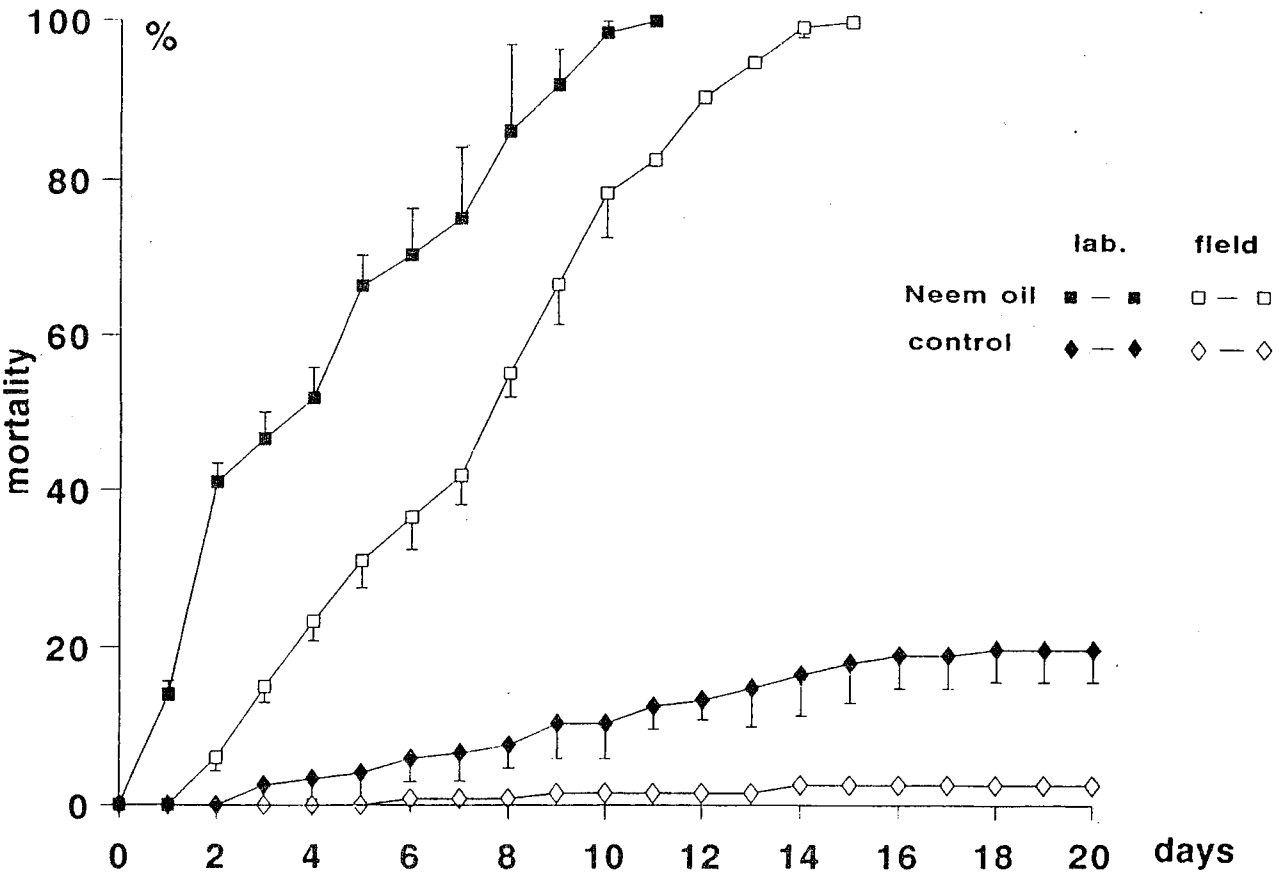


Fig. 2: Mortality rates following application of unclarified neem oil from the Trifolio-M company. The data were processed and evaluated as for Figure 1.

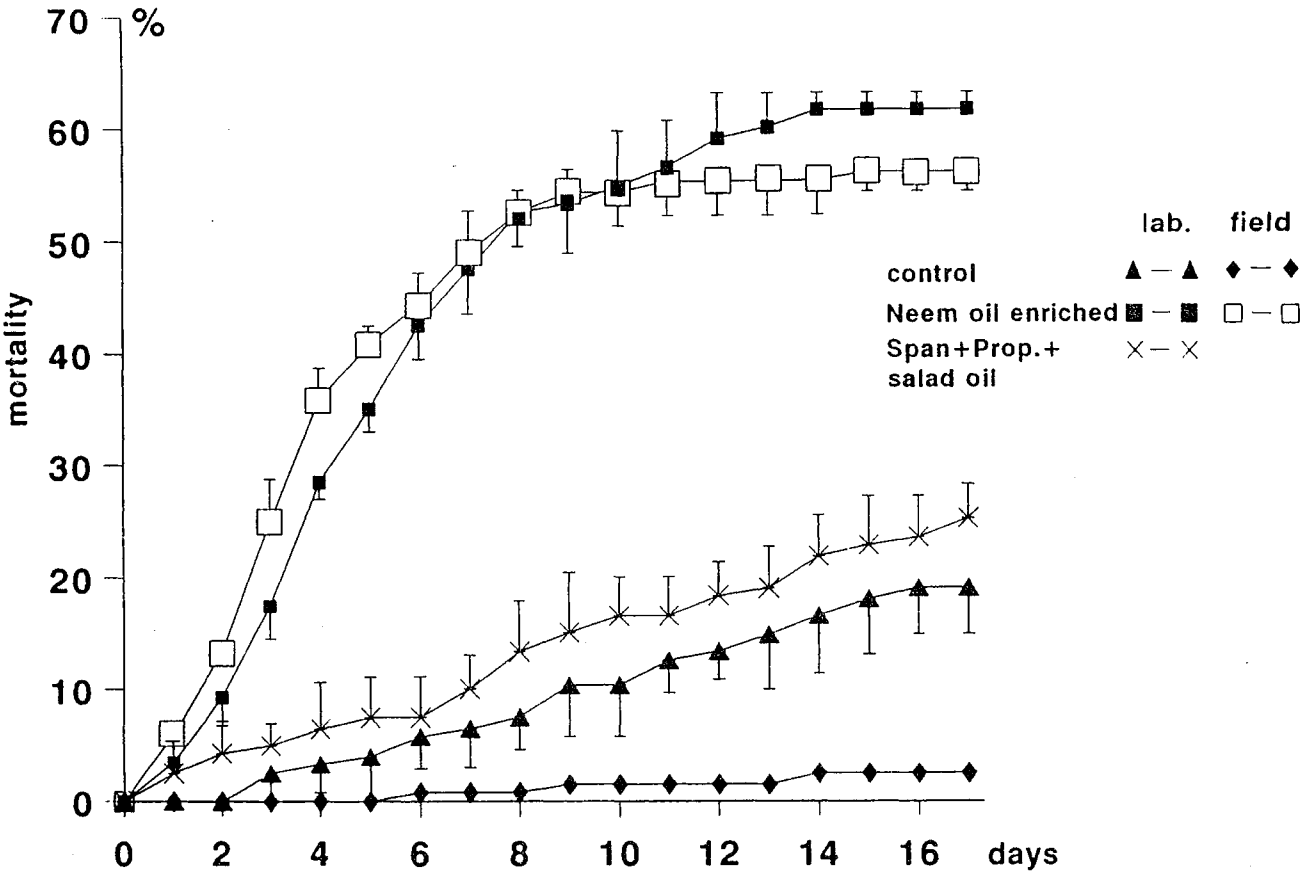


Fig. 3: Mortality rates of laboratory-reared and field-captured larvae following application of enriched neem oil (10 l per hectare) made at the University of Giessen/Germany. The data were processed and evaluated as for Figure 1.

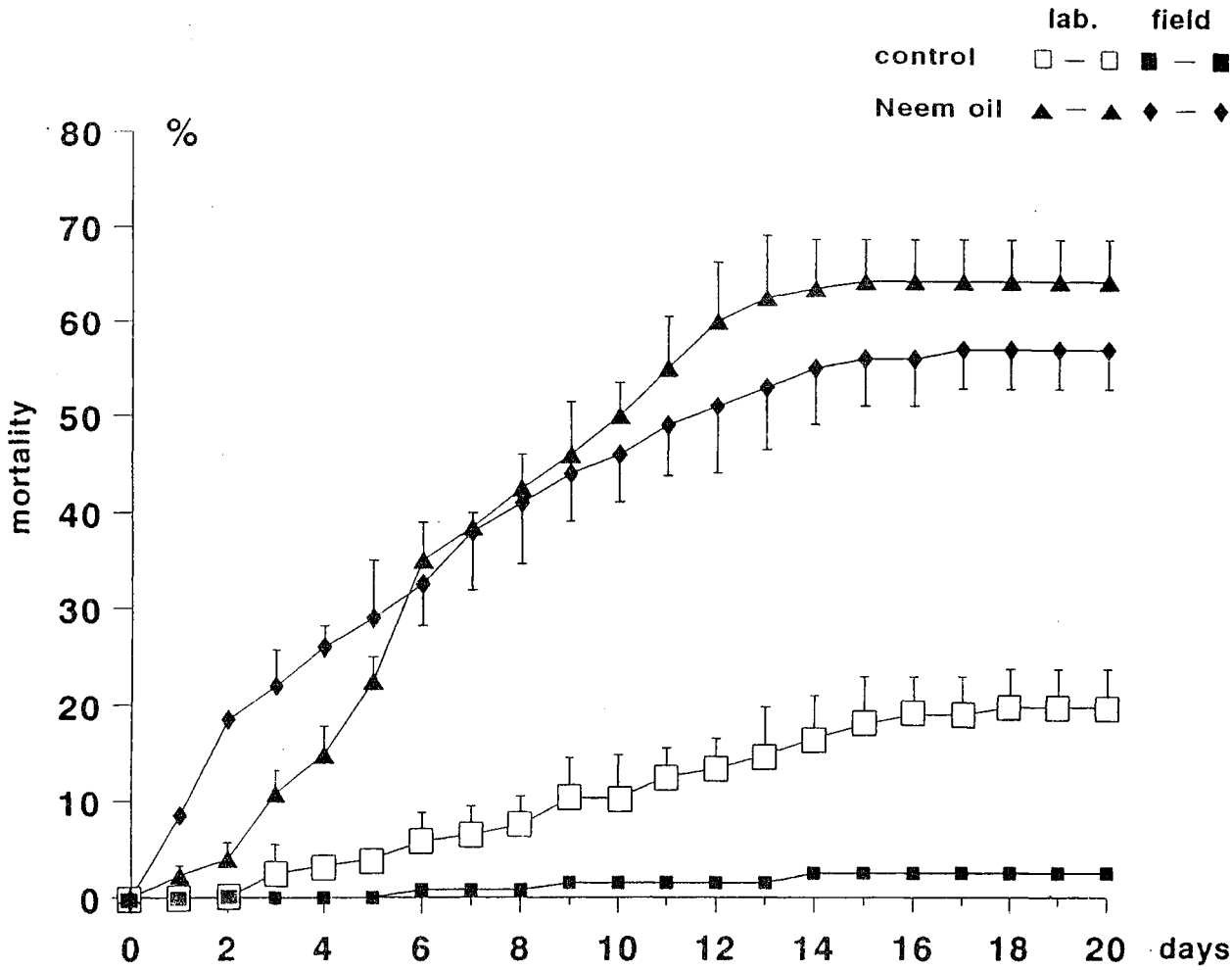


Fig. 4: Mortality rates following application of cold-pressed, pure neem oil from the University of Giessen/Germany. The data were processed and evaluated as for Figure 1.

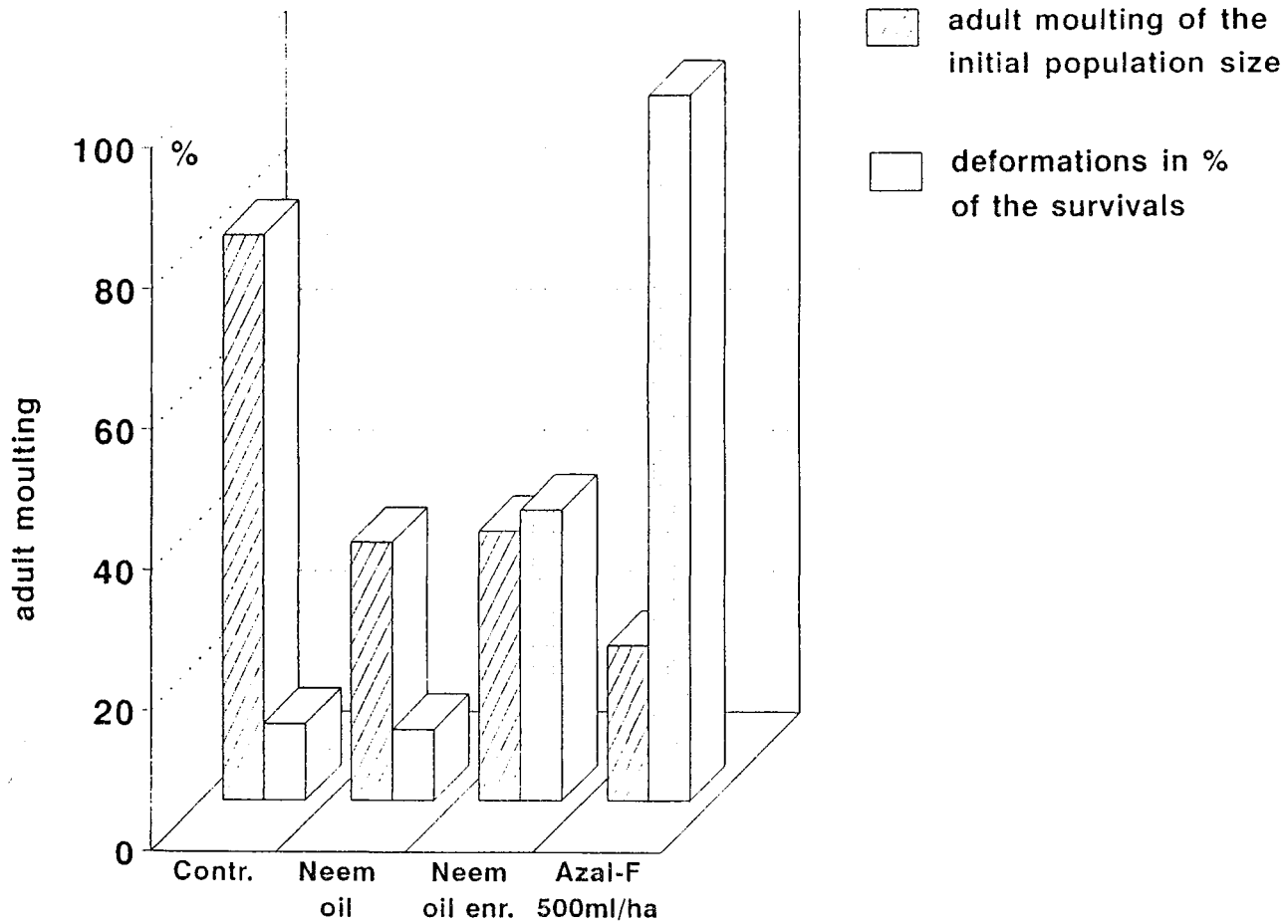


Fig. 5: Percentages of the initial populations that underwent adult molting, and the percentages of imagoes from field larvae exhibiting deformities, for different neem products.

actual practice, however, 100% mortality is only required in case of locust outbreaks where the insects have already invaded cultivated areas. For control measures in recession areas, the morphogenetic defects caused by neem products can be just as important as high mortality rates, owing to the fact that the deformed insects (Fig. 5) are then unable to leave the recession areas. Such deformations occurred, however, far less frequently than has been observed in laboratory trials with *S. gregaria* (NICOL and SCHMUTTERER 1991).

One has to bear in mind that mineral and vegetable oils, in general, are effective pesticides and repellents whose low costs, minimal health hazard make them attractive for IPM programs. Cotton seed oil were used with encouraging results against *Myzus persicae* SULZ., *Brevicoryne brassicae* L., *Spodoptera exigua* Hb., *Tetranychus* spp., *Franklinella* spp. and other insects (BUTLER and HEENBERRY 1990).

Further studies in Tamesna Desert/Niger showed that laboratory-reared larvae react sensitively to botanicals like *Mleia volkensii* too (NASSEH et al., in preparation).

The trials in Niger showed that after four weeks there was severe burning of *S.thebaica* leaves treated with enriched neem oil at the rate of 10 l/ha.

References

BUTLER, G.D. and T.J.HEENBERRY (1990): Cottonseed oil and safer insecticidal soap; effects on cotton and vegetable pests and phytotoxicity. Southwest. Entml. 15(3): pp. 257-264.

KRALL, S. and O.M.NASSEH (1990): Erforschung biologisch-integrierter Methoden zur Bekämpfung schädlicher Heu-

schrecken. Ein Projekt der GTZ, Plits 8(2), pp. 215-223.

NASSEH, O.M.; T.FRERES; H.WILPS; E.KIRKILIONIS & S.KRALL (1991): Field cage trials on the effects of enriched neem oil, insect growth regulators and the pathogens Beauveria bassiana and Nosema locustae on desert locusts in the Republic of Niger. In: Biol. Contr. of locusts and grasshoppers, pp. 311 - 320.

NASSEH, O.M.; H. WILPS and S. KRALL (1992): Neem Products - Useful Biocides for Combating the Desert Locusts Schistocerca gragaria (FORSKAL). (in press).

NASSEH, O.M.; H. WILPS and H. REMBOLD (1992): Compounds of Melia volkensii - a Schistocerca gregaria (FORSKAL) larval growth inhibitor and phase modulator. (in press).

NICOL, C.M.Y. and H. SCHMUTERER (1991): "Kontaktwirkungen des Samenöls des Niembaumes Azadirachta indica (A: JUSS.) bei gregären Larven der Wüstenheuschrecke Schistocerca gregaria (FORSKAL)". J. Appl. Ent. 111, pp. 197 - 205.

1st Workshop on the Use and Production of Neem-Ingredients
Wetzlar-Hermannstein, 18-20 June 1992

Possibilities of Vector Control

GRUNEWALD, Jorg, BOSCHITZ, Claudia E., ALLMENDINGER, Beate, URBAN, Christina
Institute of Tropical Medicine of the University of Tübingen

There is certainly a great need to control vectors of human pathogens and parasites, particularly such important vectors as: soft and hard ticks (Acari: Argasidae, Ixodidae), triatomine bugs (Heteroptera: Reduviidae), mosquitoes (Diptera: Culicidae), blackflies (Diptera: Simuliidae), sandflies (Diptera: Phlebotomidae) and tsetse-flies (Diptera: Glossinidae).

The only really efficient biological insecticides so far are *Bacillus thuringiensis var. israelensis* to control Culicidae and Simuliidae and *Bacillus sphaericus* to control Culicidae.

Very little is known about the effect of Neem-ingredients on the various developmental stages of vectors except for mosquitoes and triatomine bugs.

ZEBITZ (1986) and SCHMUTTERER (1990) observed insect growth-regulating effects of neem kernel extracts and of crude and pure azadirachtin on the larvae of various mosquito species.

Azadirachtin causes mortality and has a negative impact on the molting processes and on the fecundity of *Rhodnius prolixus* (Reduviidae) and most interesting, it inhibits *Trypanosoma cruzi* infection of its triatomine insect host as GARCIA and REMBOLD and their co-workers have shown (GARCIA *et al.* 1986, 1989; REMBOLD *et al.* 1989).

Our own studies about the effect of NeemAzal (TRIFOLIO-M GmbH, Lahnau, FRG) on *Aedes aegypti* larvae (Culicidae) showed a growth-disrupting activity against all larval stages tested. The uptake of a diet containing NeemAzal induced molting-inhibition and mortality. As with chemical larvicides sensitivity towards NeemAzal decreased with increasing age of the larvae. The calculated LC_{50}-values for larvae exposed to NeemAzal from the 2nd, 3rd and 4th instar onward were 3.3, 4.8 and 8.4 ppm, respectively. Those for the LC_{95} were 7.7, 13.8 and 22.4 ppm (BOSCHITZ 1992).

When controlling mosquito larvae with NeemAzal under tropical conditions we face two main problems:

- to achieve a satisfactory mortality rate NeemAzal has to be present in the aquatic environment of the breeding sites during the whole period of larval and pupal development.

Practice Oriented Results on Use and Production of Neem Ingredients; H. Kleeberg (ed.);

- once diluted to concentration that gives a high larval and pupal mortality NeemAzal degrades very quickly. We observed that at 30°C NeemAzal disappears from the water nearly completely within one weeks time when applied at a concentration of 40 ppm.

The control of Simuliidae, the vectors of human onchocerciasis (river blindness), is even more complicated than that of mosquitoes, since the larval stages can only develop in fast running water courses. As a result laboratory colonization and bioessays are extremely difficult due to the fact that one has to provide a continuous water flow. In the field apart from the problem of high water temperatures in the tropics the impact of the insecticide on the larvae lasts only a few seconds or minutes.

Although our first results indicate a higher susceptibility of *Simulium* larvae to NeemAzal than *Ae. aegypti* larvae, the concentration and the period of the impact on the larvae has to be much higher and longer than with other larvicides. NeemAzal showed a satisfactory effect on the larvae at a concentration of 50 mg/l over 12 hrs (ALLMENDINGER, in preparation). In comparison chemical larvicides are fully effective when applied at concentrations of 0.1 mg/l/10 min (temephos EC) and 0.1-1.0 mg/l/10 min (BTI), respectively. The WHO suggests to discharge an insecticide formulation from screening tests if the larval mortality is less than 50% at a concentration of 0.5 mg/l/10 min.

The few results on the effect of Neem kernel compounds on vectors achieved so far suggest that more work has to be done to modify the formulation and to study the effect of those formulations not only on mosquitoes, blackflies and triatomine bugs but also on all the other important vectors, particularly on ticks, sandflies and tsetse-flies.

References:

Boschitz, C., 1992: Die Wirkung von NeemAzal, einem Neembaum-Extrakt auf *Aedes aegypti* (Diptera, Culicidae). Diplom-Thesis, Faculty of Biology of the University of Tübingen, pp.68.

Garcia, E.S., Uhl, M., Rembold, H., 1986: Azadirachtin, a chemical probe for the study of moulting processes in *Rhodnius prolixus*. Z.Naturforsch. 41c, 771-775.

Garcia, E.S., Gonzalez, M.S., Azambuja, P., Rembold, H., 1989: Chagas' disease and its insect vector. Effect of Azadirachtin A on the interaction of a triatomine host (*Rhodnius prolixus*) and its parasite (*Trypanosoma cruzi*). Z.Naturforsch. 44c, 317-322.

Rembold, H., Garcia, E.S., 1989: Azadirachtin inhibits *Trypanosoma cruzi* infection of its triatomine insect host, *Rhodnius prolixus*. Naturwissenschaften 76, 77-78.

Schmutterer, H., 1990: Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann.Rev. Entomol. 35, 271-297.

Zebitz, C.P.W., 1986: Effects of three different neem seed kernel extracts and azadirachtin on larvae of different mosquito species. Z.angew.Ent. 102, 455-463.

Studies on the phytotoxicity of different neem seed extracts

G.Jakob
BBA, Institut für Pflanzenschutz im Obstbau
Schwabenheimer Str.101
6915 Dossenheim/Heidelberg

Under greenhouse condition, four different plant species namely: maize, cabbage, bean and tomato at ages of two to four weeks were examined for phytotoxic effects of different neem seed extracts. Neem seed and neem cake powder, water and alcoholic extracts and different neem oil formulations in different concentrations were used. In addition, separated neem oil components were tested on young plants. The treatments with powder were carried out by placing them in the leafwhorl of young maize plants whereas, on the other treatments the plants were totally sprayed.

As a measure for phytotoxic effects on plants, the weight, height and content of the chlorophyll were taken. Further evaluation of plant damage was assessed by using a phytotoxicity rating scale.

For result, it was found that sensitivity towards neem depended on the species of plants, their age and developmental stage. Phytotoxicity on treated plants occurred as rigid, crooked, palegreen and often smaller leaves with necrotic spots. A lower chlorophyll content and also a decrease of plant growth were recorded.

Phytotoxicity was caused mainly by neem oil whereas, water and alcoholic extracts of neem seed kernels and neem cake in concentration of 50 g/l showed no significant damage on the treated plants. The phytotoxic effects also depended on the concentration of the oil. A content of more than 2 percent of oil resulted in considerable damage. The most phytotoxic substances were found in the polar components of neem oil.

In contrast to neem cake powder, which has less oil content, neem seed powder caused phytotoxic damage in the form of necrotic and deformed leaves on young maize plants.

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Versuch der Ektoparasitenbekämpfung bei Milchschafen

W. Leupolz
Schäfereigenossenschaft Finkhof e.G.
St. Ulrich Str. 1
7954 Arnach/Bad Wurzach

Ektoparasiten verursachen bei Schafen große wirtschaftliche Schäden. Die bisherige Bekämpfung erfolgt faßt ausschließlich mit Phosphorsäureestern und in wenigen Einzelfällen mit Pyrethrum

Die Schäfereigenossenschaft Finkhof hat Alternativen zu den bestehenden Möglichkeiten gesucht und daher einen Versuch mit NeemAzal-F und Neem-Öl durchgeführt.

Eine Gruppe Milchschafe (10 Altschafe und 20 Lämmer) waren sehr stark mit Ektoparasiten (Haarlinge und Dungkäfer) befallen.

Haarlinge verursachen bei starkem Befall lebhaften Juckreiz, Unruhe, Auszupfen der Wolle und Benagen des Vlieses und damit oft beträchtliche Wollschäden.

Zur Bekämpfung dieser Ektoparasiten führten wir drei Duschbäder durch:

Duschbad 1: 100 ml NeemAzal-F mit 5% Azadirachtingehalt verdünnt mit 100 l Wasser; diese Lösung wurde mit einem Dampfstrahlgerät auf die Schafe aufgesprüht.

Duschbad 2: Ein Liter Neem-Öl mit ca. 2500 ppm Azadirachtin und 3 kg Schmierseife wurden mit 100 l Wasser gemischt. Besprühung der Schafe mit einem Dampfstrahlgerät.

Duschbad 3: 2 Liter Neem-Öl mit ca. 400 ppm Azadirachtin und 3 kg Seife wurden mit 100 l Wasser gemischt. Besprühung der Schafe mit einem Dampfstrahlgerät.

Die drei Duschbäder wurden mit einstündigem Abstand jeweils auf die gesamte Gruppe ausgebracht.

Parasitenkontrolle wurde täglich durchgeführt. Feststellung von toten Dungkäfern und Haarlingen verschwanden gänzlich. Am 5. Tag wurde ein Haarling gefunden, der vermutlich noch im Larvenstadium war.

Bei den Kontrollen konnten weder am 6., 7. und 8. Tag nach der Behandlung Ektoparasiten gefunden werden.

Zusätzlich zur Behandlung der Schafe wurden die Ställe mit der gleichen Lösung wie in Duschbad 3 ausgespritzt. Die mit dem Parasitenbefall einhergehenden, oben beschriebenen Verhaltensweisen sind seit den Duschbädern verschwunden. Auch nach 3 Monaten konnten keine weiteren Parasiten festgestellt werden.

Die Methodik der Anwendung kann eventuell durch Tauchbäder optimiert werden. Für eine genaue Einschätzung des Behandlungserfolges sollten unbedingt Bäder mit größeren Schafherden wiederholt werden.

EFFECT OF NEEM SEED EXTRACTS (*AZADIRACHTA INDICA* A.JUSS) AGAINST DOWNY MILDEW *(PLASMOPARA VITICOLA)* OF GRAPEVINE

P. ACHIMU & E. SCHLÖSSER
Wissenschaftliches Zentrum Tropeninstitut der Justus-Liebig-Universität Phytopathologie und Angewandte Entomologie Ludwigstraße 23, D-6300 Gießen

Summary

Neem seed extracts and commercial neem products (Margosan-O, Neem oil and Neem-Azal-S) had antifungal properties against P. viticola. The underside of leaves of the susceptible cv Müller-Thurgau, either in planta or detached, were sprayed with a fine mist of the neem products. Two days later, these leaves were inoculated by spray application of a suspension of 10.000 sporangia of P. viticola per ml. After 7 days of incubation, the leaf area covered by the sporulating fungus was assessed. With both systems, there was a significant reduction in disease severity by the protective treatment with an efficacy of over 90%. When neem seed extract was applied four days after inoculation with the fungus the efficacy was still over 80%, indicating a curative effect. The antifungal property of neem products could be attributed to an inhibition of the indirect germination of sporangia.

1.Introduction

The search for ways to reduce high costs, health hazards and enviromental pollution caused by the excessive use of synthetic pesticides, led researchers and farmers to try other methods to protect plants. There are many ways in which small farmers from different parts of the world, especially south east Asia, have tried to control pests through locally prepared botanical pesticides (STOLL 1989). Extracts from seeds, leaves and the bark of the neem tree (*Azadirachta indica* A. Juss) are worldwide known for their efficacy in the control of insect pests of agricultural importance. Besides their insecticidal activities, they have apparently also antifungal properties (BHOWMICK & CHOUDHARY 1982, DHARAM & SHARMA 1985, LEHMANN 1991,and ZERINGUE & BHATNAGAR 1990). In this study it was tested whether neem products have also antifungal properties against Plasmopara viticola on grapevine, a model system for downy mildews.

2. Materials and methods

2.1. Materials

2.1.1. Plants

Grapevine plants were raised from cuttings of cv Müller-Thurgau, which were kindly supplied by the Shell Forschung GmbH, Schwabenheim.

2.1.2. Commercial neem products

The following commercial neem products were tested for their efficacy against *Plasmopara viticola*: Margosan-O (from.W. R. Grace & Co.-Conn., Horticultural Products, 62 Whittemore

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Avenue, Cambridge, Massachusetts 02140, USA), Neem oil (from Dr. K. Ermel of University of Giessen, Ludwigstr. 23, D-6300 Giessen,Germany), Neem-Azal-S (from Trifolio-M GmbH, D-6335, Lahnau 2, Germany), with 0.3%, 0.08% and 0.35% azadirachtin, respectively.

2.1.3. Neem seed extracts

Neem seeds from different tropical countries, namely Togo, Niger and India, were used for preparation of aqueous neem seed extracts. According to ERMEL, et al (1986) the neem seeds contained the following concentrations of azadirachtin: Togo 3.87%, Niger 1.53% and India 3.50%.

2.1.4. *Plasmopara viticola*

The fungal population employed consisted of a mixture of many isolates, which originated from the important grape vine growing areas in the Federal Republic of Germany. This population was maintained as follows: The underside of leaves on plants of cv Müller-Thurgau was inoculated with sprays of an aqueous suspension of sporangia and then covered with transparent plastic bags to ensure the necessary high relative humidity. The inoculated plants were kept at 20-24°C with artificial illumination for a 14/10 hour photoperiod. One day p.i., the plastic bags were removed and the plants kept in a glasshouse under uncontrolled conditions with comparatively low relative humidity.

2.2 Methods

2.2.1. Preparation of extracts from neem seeds

One liter of demineralized water was added to 25g of ground neem seeds. The mixture stirred for one hour at 40°C and then filtered through four layers of cheese cloth to remove coarse materials.

2.2.2. Formulations and dilution of the commercial neem products

Neem oil needed to be formuted into an emulsion in order to have a uniform spray of the oil over the surface of the leaves. The neem oil was formulated into a mayonnaise-type emulsion. This is an oil-in-water emulsion and is water-dilutable. This emulsion is prepared using the following recipe:

Solution A:
4g emulgator mixture (ATLOX 4889 + SYNPERONIC NP10 in the ratio of 1:1)
9ml water

Solution B:
10g oil

Ensure that both solution A and B are at room temperature ca. 20°C. Add solution B to solution A slowly; If the addition is not made slowly it is highly likely that a water-in-oil rather than an oil-in-water emulsion will result. Once the oil-in-water emulsion is made, a very efficient stirrer or homogeniser with a high shear must be used to give a very fine particle sized emulsion. This was then diluted to the required concentrations by adding water. Margosan-O and Neem-Azal-S are formulated products and so, all that was needed was to dilute them with water to the required concentrations.

2.2.3. Preparation of inoculum

Inoculated leaves were collected from maintainer plants, sprayed with fine mist of demineralized water, and kept in a transparent plastic bag at 20-24°C in the dark. Twenty four hours later, the newly formed sporangia were removed with a fine painter's brush and transferred into

demineralised water.

2.2.4. Treatments and inoculation

Unless stated otherwise,the undersides of grapevine leaves were sprayed with a fine mist of the aqueous neem seed extracts or commercial neem products, respectively. The check was sprayed with a fine mist of demineralized water. Forty eight hours later, suspensions of about 10.000 sporangia/ml of *P. viticola* were uniformly applied on the dried underside leaf surfaces with a mist-sprayer. Detached leaves were kept on moistened filter discs in Petri dishes. Inoculated plants were covered with transparent plastic bags for seven days. In both systems, the incubation was at 20-24°C with a 14/10 hour photoperiod of artificial illumination.

2.2.5. Evaluation

Each variant consisted of 10 detached leaves, considered as replications, or two grape vine plants with 5-6 fully expanded leaves, which were all treated. Seven days p.i., the leaf area with sporulation was assessed according to the method described by KRANZ (1970) as in previous investigation (ACHIMU & SCHLÖSSER 1991)

3. Results

3.1 Effect of different neem products against *P. viticola*

When Neem-Azal-S and Neem oil were applied in an aqueous dilution of 1:150ml, the surface area covered with sporulation seven days after inoculation was reduced by more than 99% in comparison to the control (Tab. 1).

Tab. 1: Effect of commercial neem products against *P. viticola* on detached leaves.

Treatment	% of leaf area with sporulation	% efficacy
check	62.1[c]	
NO[a] 1:150	0.3	99.5
NAS[b] 1:150	0.1	99.8

a = Neem oil
b = Neem-Azal-S
c = average from 10 replicates each of 2 experiments

3.2 Effect of aqueous neem seed extracts against *P. viticola*

After the first experiments with commercial neem products and with blanks of the commercial products, it was not clear whether the inhibiting effect against the pathogen was due to the active ingredients of the neem or due to the preservatives of the products. Therefore experiments were conducted using aqueous neem seed extracts, which contained no preservatives. The sporulation was similarly inhibited by more than 90% in both experiments with leaves placed in Petri dishes and experiments done in planta (Tab. 2).

Tab. 2: Effect of neem seed extracts (Togo 1990 supply) against *P. viticola*.

Treatment	% of leaf area with sporulation	% efficacy
detached leaves		
check	50.0[b]	
NSE[a]	0.4	99.1
in planta		
check	42.4[b]	
NSE[a]	0.2	99.6

a = Neem seed extract
b = average from 10 replicates each of 2 experiments

Tab. 3: Effect of extracts prepared from neem seeds obtained from different geographical locations against *P. viticola* on detached leaves.

Treatment	% leaf area with sporulation	% efficacy
check	50.0[b]	
NSE[a] (Togo 1986)	4.8	90.4
NSE (Togo 1990)	1.2	97.6
NSE (Niger 1991)	17.5	65.0
NSE (India 1991)	1.6	96.8

a = Neem seed extract
b = average from 10 replicates each of 2 experiments

When extracts of neem seeds from different countries were compared (Tab.3), the preparations Togo 1986, 1990 and India 1991 had about the same efficacy, while the preparation Niger 1991, which had also the lowest azadirachtin content, was significantly less effective.

3.3 Effect of dilution of neem products and neem seed extracts on the efficacy of neem against *P. viticola*.

Commercial neem products and the neem seed extracts were diluted and tested for antimycotic effectiveness. Neem oil and Neem-Azal-S were still lightly effective at a dilution of 1:450, while Margosan-O had only an efficacy of 50% at the recommended dosage of 1:150 for insect pest control.

Tab. 4: Effect of dilution on the efficacy of commercial neem products against *P. viticola* on detached leaves.

Treatment	% leaf area with sporulation	% efficacy
check	62.1[d]	
NO[a] 1:150	0.3	99.5
NO 1:300	0.8	98.7
NO 1:450	1.3	97.9
NAS[b] 1:150	0.1	99.8
NAS 1:300	0.1	99.8
NAS 1:450	0.9	98.6
MSO[c] 1:150	36.2	41.7
MSO 1:300	50.0	24.2
MSO 1:450	50.0	24.2

a = Neem oil,
b = Neem-Azal-S
c = Margosan-O
d = average of 10 replicates each from 2 experiments

Tab. 5: Effect of dilution on the efficacy of neem seed extracts (Togo 1990) against *P. viticola* on detached leaves.

Treatment	% leaf area with sporulation	% efficacy
check	50.0[b]	
original conc.	1.8	96.4
NSE[a] 1:1	5.7	88.6
NSE 1:4	31.8	36.4
NSE 1:9	40.5	19.0

a = Neem seed extract
b = average of 10 replicates each from 2 experiments

3.4 Persistence of antifungal activity

In order to determine how long neem remains active once sprayed on the plant, the following experiments were carried out under green house conditions: Grapevine leaves on whole plants were sprayed with neem extract and 2, 4, 9 and 14 days later they were inoculated with P. viticola,

respectively. The antifungal activity proved to be rather stable (Tab. 6) and persisted 14 days without appreciable loss in effectiveness.

Tab. 6: Effect of time interval between treatment and inoculation on the efficacy of neem seed extract (Togo 1990) against *P. viticola* in planta.

Treatment	% leaf area with sporulation	% efficacy
check	50.0[a]	
2 days	0.1	99.8
4 days	0.1	99.8
9 days	0.1	99.8
14 days	0.1	99.8

a = average of 10 replicates from 2 experiments

3.5 Curative effect against *P. viticola*

In order to test whether neem seed extracts have also a curative effect, inoculated leaves were treated 1-4 days p.i. (Tab. 7).

Tab. 7: Curative effect of neem seed extract (Togo 1990) against P. viticola in planta.

Treatment after days	% of leaf area with sporulation	% efficacy
check	50.0	
0 d	0	100.0
1 d	2.7	94.6
2 d	2.7	94.6
3 d	4.8	90.4
4 d	7.9	84.2

a = average of 10 replicates from 2 experiments

The neem seed extract had a remarkable curative effect with an efficacy of still 84.2% four days p.i.

3.6 Mode of action of neem products against *P. viticola*

In order to obtain information about the mode of antifungal action, sporangia of P. viticola were treated with various neem products to test their effect on the indirect germination. In the first part of this investigation the sporangia were kept in the neem suspensions at room temperature. Twelve

hours later the germination of sporangia wa microscopically evaluated (Tab. 8).

Tab. 8: Effect of commercial neem products on the indirect germination of P. viticola

Treatment	% Germination
check	95.5[d]
NAS[a] 1:150	3.2
MSO[b] 1:150	1.8
NO[c] 1:150	1.5

a = Neem-Azal-S
b = Margosan-O
c = Neem oil
d = average of 10 replicates each from 2 experiments

All products inhibited sporangia germination.

In the second part of the investigation the sporangia incubated for 12 hours in solutions of the neem products were washed with demineralized water by repeated cycles (4 times) of centrifugation at 5000rpm in order to remove the neem products. After resuspension in demineralized water the sporangia were microscopically evaluated for indirect germination twelve hours later (Tab. 9).

Tab. 9: Germination of pre-treated but washed sporangia of P. *viticola*

Treatment	% Germination
check	98.0[a]
NAS 1:150[b]	3.0
MSO 1:150[c]	3.5
NO 1:150[d]	2.5

a = average of 10 replicates each from 2 experiments
b = Neem-Azal-S
c = Margosan-S
d = Neem oil

Although the water soluble neem products were most likely removed by the repeated washings, the high degree of inhibition of sporangia proved to be permanent. Thus, a prevention of zoospore formation and/or release must be considered as a mode of action of the antifungal components in the neem preparations.

4. Discussion

Under laboratory and green house conditions, commercial neem products as well as extracts of neem seeds from different countries (Togo, Niger and India) proved to be highly effective against *P. viticola*. There are also indications of a good curative effect. The efficacy is due to an inhibition of the indirect germination of sporangia by preventing zoospore formation and/or release. The process of sporangia germination is quite sensitive to osmotic pressure (GEISLER 1959) which can be completely inhibitory at a certain level. The existing osmotic pressure of the neem preparations was, however, not the cause of their efficacy. When the pre-incubated sporangia were washed free from the neem products and placed in demineralized water, they did not germinate, a sign of irreversible damage.

The insecticidal components of neem extracts, such as azadirachtin, are rather unstable and readily decomposed in light (SCHMUTTERER 1989). The hitherto unknown antifungal components are, however, rather stable. After an exposure of 14 days on leaf surfaces they were still fully effective. This agrees with the finding of LEHMANN (1991) that the antifungal components are apparently different from azadirachtin and related substances. The high efficacy of neem products agaainst *P. viticola* under laboratory and green house conditions has to be validated by applications in vineyards. Unfortunately, this was not yet possible because there was hardly any *P. viticola* present in the years 1990/1991.

An application of neem seed extracts could be a promising alternative in the control of fungal pathogens for small farmers in developing countries. In tropical areas neem seeds can be easily produced, on trees near the house, allowing a homemade preparation of neem seed extracts with both, insecticidal and antifungal properties. Besides, neem extracts are "harmless to mammals and man and enviromentally sound" (SCHMUTTERER 1989).

5. References

ACHIMU, P. & E. SCHLÖSSER (1991): Control of *Plasmopara viticola* with compost filtrates. Med. Fac.Landbouww. Rijksuniv. Gent, **56**, 171-178.

BHOWMICK, B. N. & B. K. CHOUDHARY (1982): Antifungal activity of leaf extracts of medicinal plants on *Alternaria alternata* (Fr.) Keissler. Indian bot. Reptr. **1**, 164-165.

DHARAM, V. & R. K. SHARMA (1985): Efficacy of Fungicides XXIX. Studies on the fungicidal properties of Neem oil. Indian J. Plant Pathol. **3**, 241-242.

ERMEL, K.,E. PAHLICH & H. SCHMUTTERER (1986): Azadirachtin content of neem kernels from different geographical locations, and it's dependence on temperature, relative humidity and light. Proc. 3rd Int. Neem Conf., Nairobi **1986**, 171-184.

GEISLER, G. (1959): Zoosporenbildung und - Keimung bei Plasmopara viticola in Abhängigkeit vom osmotischen Wert. Viti **2**, 40-44.

KRANZ, J. (1970): Schätzklassen für Krankheitsbefall. Phytopath. z. **69**, 131-139

LEHMANN, W. (1991): Fungizide Inhaltsstoffe aus den Kernen des Neembaumes (*Azadirachta indica* A. Juss) - Ihre Gewinnung und Wirkung auf Wurzelbranderreger der Zuckerrübe. Dissertation. Göttingen.

SCHMUTTERER, H. (1989): Proc. of the Feb. 1989 Integrated pest management in tropical and subtropical cropping systems, Roundtable Bad Dürkheim, vol. III, 686.

SCHMUTTERER, H. (1990): Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann. Rev. Entomol. **35**, 271-297.

STOLL, G. (1989): Botanical pesticides. The sustainable Agriculture Newsletter, **1**, 1-16.

ZERINGUE, H. J. Jr. & D. BHATNAGAR (1990): Inhibition of aflatoxin production in *Aspergillus flavus* infected cotton bolls after treatment with neem (*Azadirachta indica*) leaf extracts. J. American Oil Chem. Society. **67**, 215-216.

6. Acknowledgement

This investigation was supported by a grant from the Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) through the German Academic Exchange Service (DAAD).

NEEM AZAL/NEEM AZAL F IN THE AQUATIC ENVIRONMENT

Peter Schröder , IfAH Institut für Angewandte Hydrobiologie Konstanz
Grossherzog-Friedrich-Strasse 2A, D(W) 77750 Konstanz 16
☎ (0049) - 7531 - 44439 / (0049) - 7531 - 44890

1. Introduction

We were invited by the Seychelles' Ministry of Tourism and Transport to develop and carry out a programme for control of blood sucking gnats (Ceratopogonidae) by non-chemical methods. As in some other tropical and subtropical areas worldwide, the aggressive species of *Leptoconops* , the biting "sandflies" are a serious and increasing problem at many public beaches which hinder tourism.

Despite of regular treatment of the public beaches using chemical insecticides, the biting rate of "sandflies" has not been reduced to an acceptable level. First control programmes in the Seychelles used DDT, later less persistent insecticides like Malathion and Dizinon were employed.

The concept of the programme is based on both an understanding of the biology and ecology of the species concerned, and the results from succesfully performed field and laboratory experiments in Florida, Caribicis, French Polynesia, Madagascar and other countries. It proposes the application of hydrological methods and Neem-Azal, and Neem-Azal F resp.

We suggested a three - step - programme:

- 1. **A one month - feasibility study** on the microdistribution and biology of *Leptoconops spinosifrons* and first field experiments for sandfly control by application of Neem as well as effects on the non-targed fauna.

- 2. **A three months - pilot phase** for test spraying on a selected beach section under controlled conditions.

- 3. **A longterm routine application** in large areas of the successful alternative non-chemical methods which were adapted to the individual local conditions. These methods will initially support the effects of chemical insecticides, and can later replace the latter completely.

In addition to field studies in the Seychelles, laboratory experiments have to be carried out at the Institute in Constance in close cooperation with Trifolio GmbH in Lahnau to analyze stability, adsorption/desorption processes of Neem Azal powder and Neem Azal F under special conditions of the marine environment (salinity, coral sand) as well as their effects on the non-targed aquatic fauna.

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

2. Results

2.1. Field studies on environmental conditions and distribution of *Leptoconops spinosifrons* in the Seychelles

In 1991 Inge Werner, Dr. Wolfgang Joost and I carry out a feasibility study on invitation by the Ministry of Tourism & Transport. (Fig. 1)

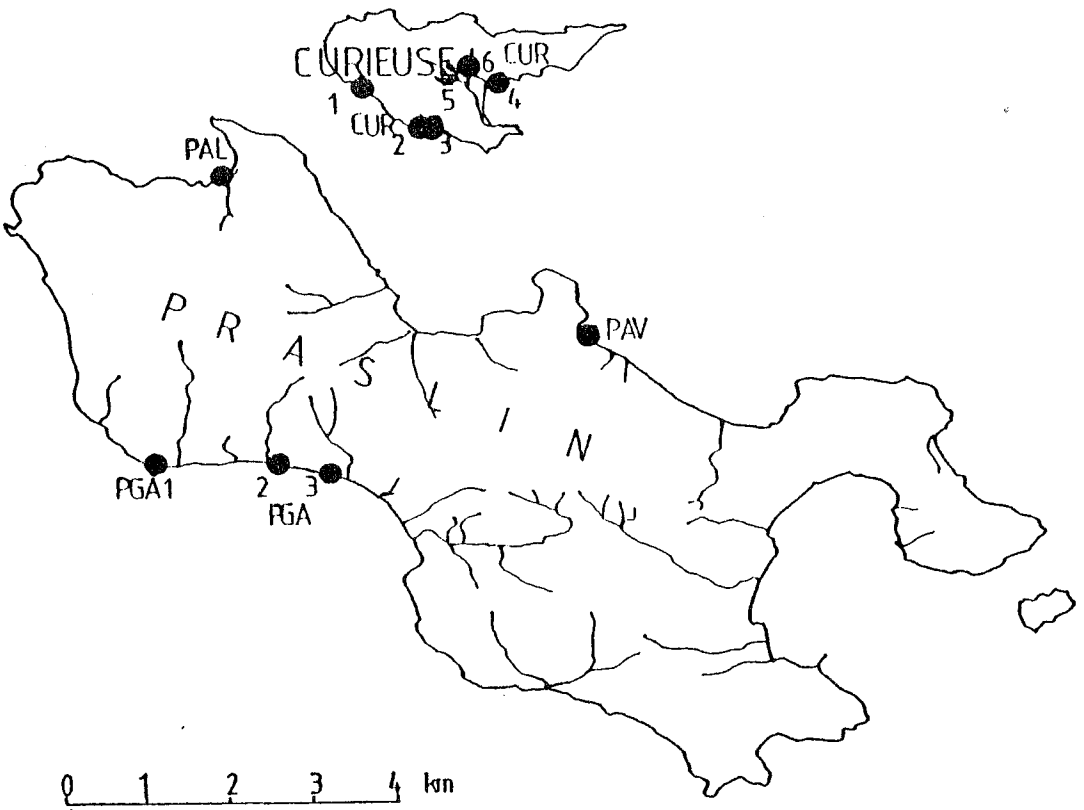


Fig. 1: Location of Praslin and Curieuse Islands of the Granitic Seychelles, where beaches were investigated for larvae of *Leptoconops spinosifrons* .

In the Seychelles, *Leptoconops spinosifrons* inhabits the upper beach zone which is only flooded by high tides . The larvae and pupae live in a zone a few meters wide between high tide mark and the landward fringe of the beach. LAURENCE & MATHIAS (1972) and REYNOLDS (1972) found that the breeding area include parts of the beach covered by beach morning glory *Ipomea pes-caprae* and associated grasses, but also included pure coral sand, and sand covered by seaweed and other sea drift. Female *Leptoconops* depend on a blood meal for the completion of their ovarian cycle. The larvae can hatch within some hours after brief flooding by spring tide (DUVAI et al. 1974), esp. under conditions of heavy tropical rain showers. This is because the larvae develop at 8 - 15 ppt salinity which is less than half the salinity of sea water. The larvae live 0 - 30 cm below the sand surface. Mature pupae and the newly emerged sandflies concentrate immediately below the sand surface. Emergence time and flight activity depend on the time of the day (light conditions) and tidal phase.

The females do not move very far along seaside; they can penetrate in the hinterland as far as half a mile if there is no arboreal vegetation or thick bushes (DUVAL et al. 1974).

Several beaches were investigated for larvae of *Leptoconops spinosifrons* by direct sieving or salt flotation of the sand samples, while the adults were caught by light traps using battery-operated Neon tubes. We did not find any larvae of *Leptoconops spinosifrons*. Only adults were observed by ourselves on some beaches. These observations were in accordance with findings of the Entomological Unit of the Ministry of Health on Praslin. The scarcity of *Leptoconops spinosifrons* can be attributed to the very dry summer of 1991. Hatching of *Leptoconops* eggs only takes place at a specific sand humity. If the conditions are unfavorable, the eggs can survive dry seasons up to 6 months without being flooded (DUVAL et al. 1974).

2.2. Neem-Azal F effects on *Leptoconops spinosifrons* and the associated non-targed fauna in the Seychelles

Experiments with Neem-Azal F were carried out on its effects on *Leptoconops spinosifrons* and the associated fauna. Molluscs were determined by ABBOTT & DANCE (1990).The investigated taxa and experimental conditions are described below, results are summarized in table 1.

***Leptoconops spinosifrons* (Ceratopogonidae).** At Grand Anse Beach (PGA 2 in Fig. 1) sandflies and associated fauna as Microlepidoptera, Empididae, Dolichopodidae, Formicidae and Curcullionidae were caught by light traps. Neem-Azal F was sprayed on water in a white laboratory trap, and a battery-operated neon tube placed abov the tray. The insects got stuck on the water surface and died immediately. We assume that Neem Azal F forms a lipophilic surface layer which hinder the insects from escaping.

Mosquitoes (Culicidae) were caught from crab holes on Curieuse Island (CUR 3 in Fig. 1). Twenty adult mosquitoes were placed into an experimental cage where Neem-Azal F was to be applied. A solution of 1:1000 Neem-Azal F : water was sprayed into the cage. The mosquitoes died immediately after contact with Neem-Azal F aerosol. Mosquito larvae were collected from a rock pool on Curieuse Island (water temperature: 33.3°C, conductivity: 140 µS/cm). Twenty mosquito larvae (last instar) were placed into each experimental beaker containing 400 ml of Neem-Azal F solution at various dilutions and into a control containing 400 ml of freshwater. In a first experiment, larvae were exposed to dilutions of Neem Azal F : water between 1: 200 and 1: 833 (250 to 60 ppm Azadirachtin). At 125 and 250 ppm all mosquitoe larvae died within 14 hours, while 95 % of the larvae were killed at 60 ppm after 20 hours (Fig. 2). Some days after, the experiment was repeated with concentrations of 30 to 125 ppm Azadirachtin and showed similar results (Fig.3).

***Nassarius arcularius plicatus* (Fam. Nassaridae)** is a marine snail common on sandy intertidal zone of the Granitic Islands of the Seychelles. These snails were collected from Grand Anse beach in front of the Flying Dutchman Hotel. Each three snails were placed into a beaker with 250 ml pure sea water from control or into Neem-Azal F diluted 1: 500 into sea water (1: 500). In the control beaker, the snails

showed normal reactions until released. In the beaker containing 1:500 Neem-Azal F, all 3 snails closed their opercula immediately and showed no further reactions even after replacing into pure seawater. A second experiment was performed applying Neem-Azal F in dilutions of 1 : 500, 1 : 2000 and 1 : 8333. At lowest concentration all 4 snails moved around for about 5 min, then they showed no further reactions without being contracted. After three hours 2 snails made orientation movements (table 1).

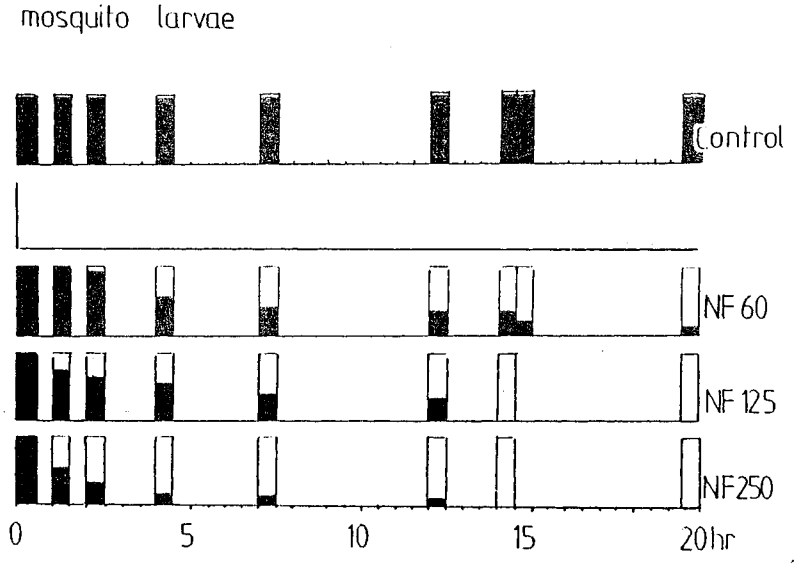


Fig. 2: Mosquito experiment with larvae from Curieuse rock pool on 20th Nov. 1991. Initially, twenty larvae (total bar) were placed into each experimental beaker. Black bars shows the proportion of surviving larvae in control and different dilutions of Neem-Azal F: freshwater with time.

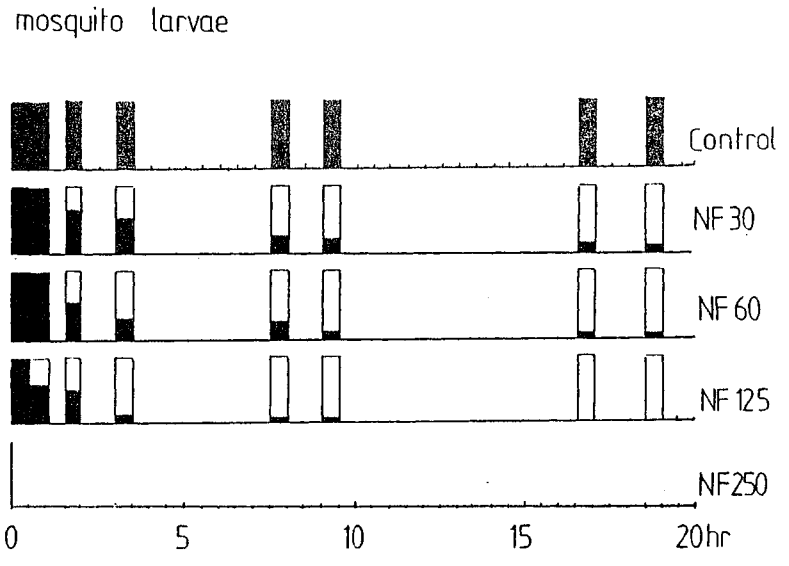


Fig. 3: Mosquito experiment with larvae from Curieuse rock pool on 22th Nov. 1991.

***Littorispina* spec. 1** , a semiterrestric species was collected at the supratidal zone of Anse St.Joseph (CUR 2) on Curieuse Island. The snails in the control were sprayed with freshwater and showed normal reactions during several hours of observation. A second group was sprayed with Neem-Azal F aerosol (dilution 1:1000). These snails retracted their feet immediately and showed no further reactions.

***Littorispina* spec. 2**, a marine intertidal species was taken from Grand Anse /Praslin (Fig. 1) and tested at dilutions of Neem-Azal F : sea water between 1: 1000 and 1: 50000. Only 10% survived the first 30 min after they were exposed to the lowest Neem-Azal F test concentration , while all snails died within the first 15 min at higher levels of Neem-Azal F (table 1).

***Nerita plicata* (Neritidae)**, a marine supratidal species was collected from Grand Anse /Praslin (PGA 2, Fig.1). Seven snails were exposed to Neem-Azal F aerosol (1: 1000). They contracted immediately and showed no further reactions.

Isopods were collected from decaying material of seaweed at Anse St.Joseph on Curieuse Island (CUR 2) and placed into experimental boxes. One control box containing 20 isopods was sprayed with freshwater. Another box containing 20 isopods was sprayed with Neem-Azal F aerosol (dilution 1:1000). The animals in both boxes showed normal reactions and activity after the liquid had evaporated.

Fiddler Crabs (*Uca* spec., Decapoda) were collected from a mangrove swamp in Pickwood River estuary on Praslin . Five crabs were placed into each experimental box containing (a) 2 cm layer of sand moistened with sea water; b) 2 cm layer of sand moistened with Neem-Azal F-seawater solution (1:1000); (c) 2 cm layer of sand moistened with Neem-Azal F-seawater solution (1:1000) after the crabs had been placed on the sand. In experiment (b), the crabs immediately developed foam in front of their mouth parts and then tried to dig holes in the 2 cm sand layer. In experiment (c) digging and foam development began at the same time. The crabs obviously tried to get rid of the Neem. The control animals did not show any abnormal behaviour and no development of foam. After 3 hours, the animals were buried in sand and still alive, without foam.

Table 1

Experiments with Neem Azal F in the Seychelles November 1991

Taxon (total indiv.)	(Neem Azal F dilutions) / Azadirachtin concentrations ppm) Control 0	1:50000 1	1:10000 5	1:50000 10	1:20000 25	1:10000 50	1:833 60	1:500 100	1:400 125	1:200 250ppm
Adult sandflies *L.spinosfrons*						■				
Adult mosquitoes Culicidae (20)						■				
Mosquitoe larvae Culicidae (80) 22 hours	○						▨		■	■
Mosquitoe larvae Culicidae (80) 19 hours	○				30ppm ▨		▩		■	
Marine snails *N.arcularius plic.* (20) 3 hours	○			60ppm ▨	▨			■		
Semiterrestric snails *Littorispina* sp.1 (60) 3 hours	○					■				
Intertidal snails *Littorispina* sp.2 (80) 30 min	○	▨	■	■		■				
Marine snails *Nerita plicata* (7) 30 min						■				
Marine isopods (40) 3 hours	○					○				
Fiddler crabs *Uca* spec. (15) 3 hours	○					⊘ 1) ⊘ 2)				

Explanations:

○ = all animals survived, normal behaviour

⊘ = all animals survived, abnormal reaction (retraction, orientation movements, developing of foam)

1) sand moistened with Neem- Azal F areosol **before** crabs had been placed on the sand ⇨ crabs immediately developed foam in front of their mouth= parts and then tried to dig holes in the 2 cm sand layer.

2) sand moistened with Neem-Azal F aerosol **after** crabs had been placed on the sand ⇨ digging and foam development began at the same time.

▨ = 50 % survived

▨ = 10 % survived

▩ = 5 % survived

■ = no animals survived

2.3. Test spraying of Neem-Azal F on Anse Amitié/Praslin (Seychelles)

By kind assistance of the Entomological Unit of the Ministry of Health, a beach section m of Anse Amitié just opposite Praslin Airport were test-sprayed twice with a solution of Neem-Azal F. This beach was selected because it had not been sprayed before with Diazinon, although biting activity of *Leptoconops spinosifrons* was recorded. 40 liters of Neem-Azal F at a dilution of 1:1000 (corresponding to 50 ppm Azadirachtin) were applied to the upper beach area from high tide level to approx. 3 meters into the area covered by beach morning glory *Ipomea pes-caprae* . The Neem solution showed good spraying characteristics (not sticky, no foam) and did not smell. Its infiltration into the sand is good as tested before in small-scale infiltration experiments with test boxes at Grand Anse/Praslin.

2.4. Neem Azal laboratory experiments on aquatic fauna (Germany)

Laboratory experiments were carried out at the Institute for Applied Hydrobiology Constance in March 1992 to investigate letal and subletal effects of Neem-Azal F as well as Neem Azal powder on non-targed aquatic fauna of subtropical and temperate zones. The species were determined by ENGELHARDT (1983) and FECHTER & FALKNER (1989).

Neem- Azal F (approx. 5% Azadirachtin) and the pure Neem- Azal powder (approx. 30 % Azidirachtin) were diluted into water taken from aquariums for precultiviations of the test organisms. The experiments were carried out with pure aquarium water for control and test media of approx. 1 , 5, 10 and 50 ppm Azidirachtin. In the following these diutions were named as NF 1, 5, 10, 50 (for Neem-Azal F) and NP 1, 5, 10, 50 (for Neem-Azal powder).

The test organisms were observed for 48 hours under semi-natural conditions of light/darkness and water temperatures between 16 - 20^{o}C. The surviving rates and subletal effects on behaviour are shown in Fgs. 4 - 9 . Water samples and ethanol - fixed animals were stored for later residue control and Azidirachtin contents by Trifolio GmbH in Lahnau.

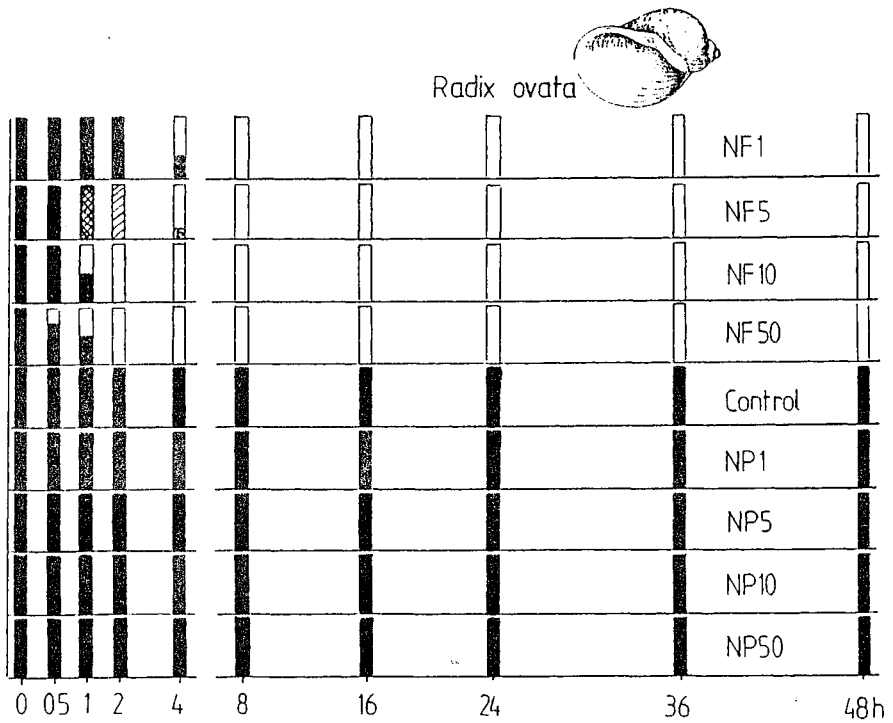


Fig. 4: Surviving proportions of the freshwater snail *Radix ovata* (total of 61 individuals) in control and under permanent exposition of various concentrations of Neem-Azal F (NF 1 - 50) and Neem-Azal powder (NP 1 - 50) within 48 hours. ■ = proportion of surviving animals; ▩ = animals showing retarded movements ▨ = animals loosing mucus ⁘ = inactive, immobile animals.

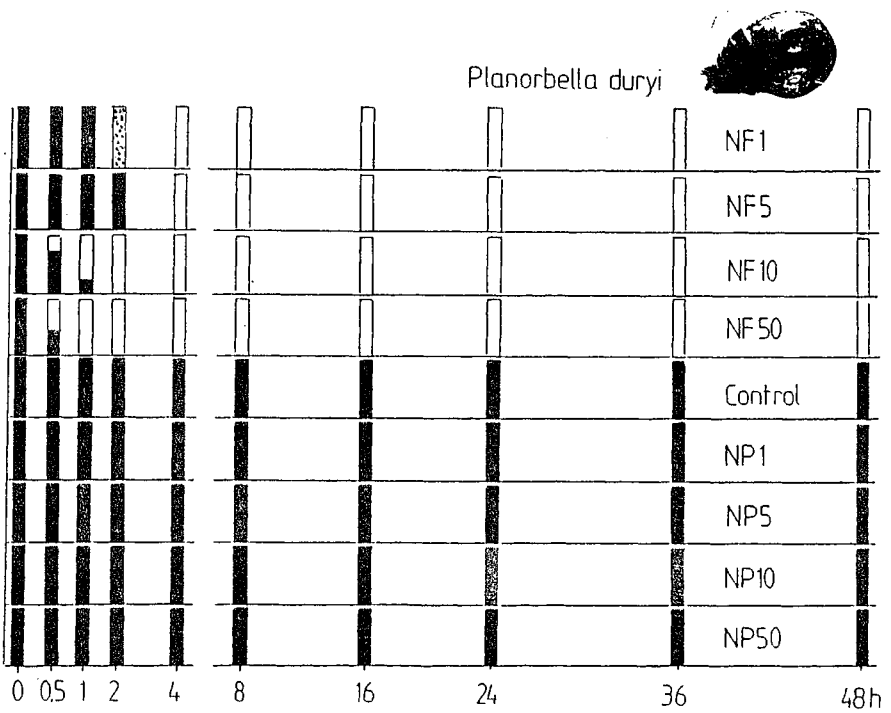


Fig. 5: Surviving proportions of the subtropical freshwater snail *Planorbella duryi* (total of 32 individuals) in control and under permanent exposition of various concentrations of Neem-Azal F (NF 1 - 50) and Neem-Azal powder (NP 1 - 50) within 48 hrs. ■ = proportion of surviving animals; ⁘ = inactive, immobile animals.

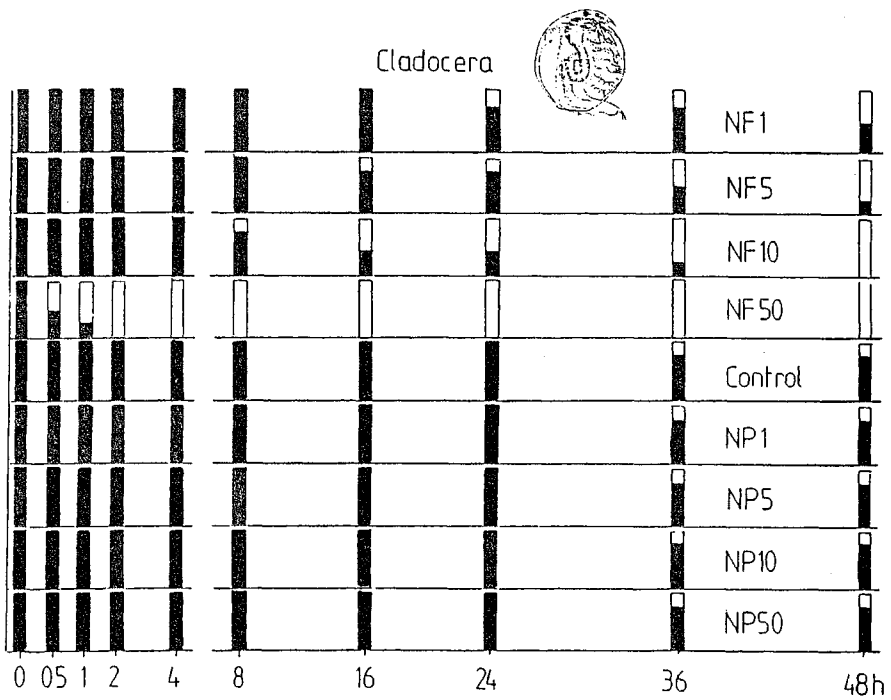


Fig. 6: Surviving proportions of Cladocera (probably *Chydorus sphaericus*) from a forest pond (total of approx. 450 individuals) in control and under permanent exposition of various concentrations of Neem-Azal F (NF 1 - 50) and Neem-Azal powder (NP 1 - 50) within 48 hours. ▓ = proportion of surviving animals.

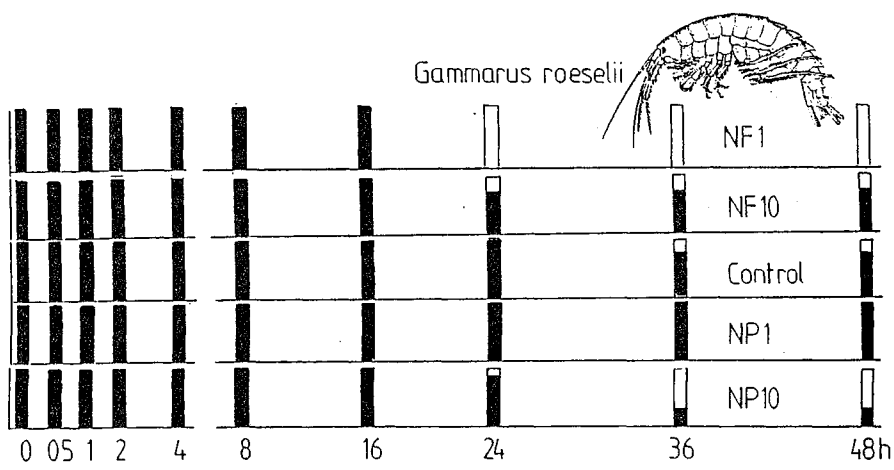


Fig. 7: Surviving proportions of the freshwater amphipod *Gammarus roeselii* from River Rhine below Lake Constance (total of 26 individuals) in control and under permanent exposition of various concentrations of Neem-Azal F (NF 1 - 10) and Neem-Azal powder (NP 1 - 10) within 48 hours. ▓ = proportion of surviving animals.

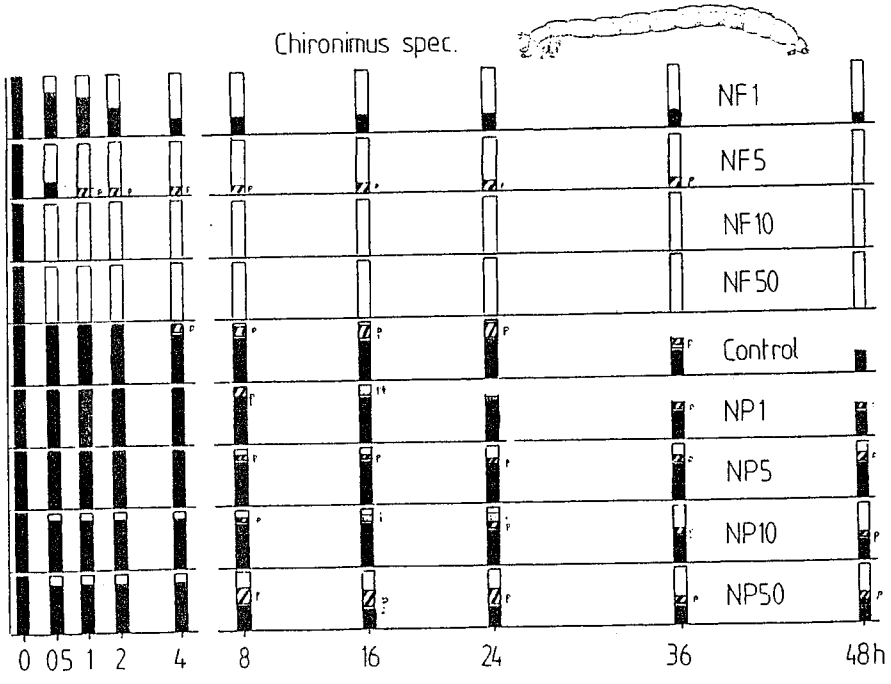


Fig. 8: Surviving proportions of red water midges of the *Chironimus thummi*-Group (total of 96 individuals) in control and under permanent exposition of various concentrations of Neem-Azal F (NF 1 - 50) and Neem-Azal powder (NP 1 - 50) within 48 hours. ■ = proportion of surviving larvae ; ▨ p = proportions of living pupae ⬚ i = proportions of emerged imagos.

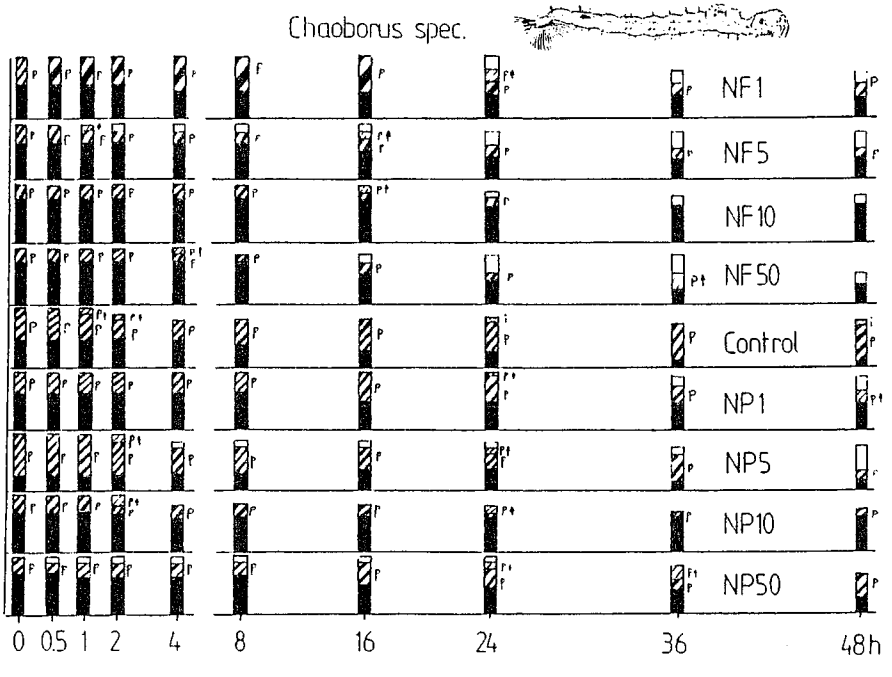


Fig. 9: Surviving proportions of the phantom midges *Chaoborus* spec. from garden ponds (total of 61 individuals) in control and under permanent exposition of various concentrations of Neem-Azal F (NF 1 - 50) and Neem-Azal powder (NP 1 - 50) within 48 hours. ■ = proportion of surviving larvae ; ▨ p = proportions of living pupae; ▨ p⇧ = proportions of pupal skins, imagos emerged ⬚ i = proportions of imagos remaining on water surface after emerging.

3. Conclusions

3.1. The natural insecticide Neem-Azal F has been successfully applied to aquatic blood-sucking Diptera in the Seychelles (e.g. mosquito larvae and adults). Neem is thought to be a promising potential tool against mosquitoes. For adult *Leptoconops spinosifrons*, both the spraying test (Neem - Azal F aerosol, dilution 1: 1000) and a Neem-Azal F water mixture (in combination with light traps) proved to be lethal.

3.2. Both the marine and terrestrial non-targed beach fauna (insects, snails and crustaceans) are affected by Neem - Azal F. Whereas molluscs showed strong to lethal effects, all crustaceans tested survived our experiments.

3.3. In such short-term experiments, the reactions shown by Nematocera larvae cannot be attributed to antihormone effects. We assume that the observed mortality of mosquitoes , sandflies and the associated faunas were caused by toxic effects of the Neem Azal F formulation. These assumptions were hardened through further laboratory experiments in Germany, when Neem-Azal F and Neem-Azal powder were tested on the non-targed fauna.

3.4. The toxic effects on behaviour and surviving rates of Neem-Azal F and pure Neem -Azal powder were tested on non-targed aquatic fauna within short-time experiments of 48 hours in IfAH laboratory in Constance. Applications of Neem-Azal F showed similar effects on aquatic snails and crustaceans as in the Seychelles, while all animals survived if dilutions of the Neem - Azal powder were applied.

3.5. Water midges (Chironomidae) showed similar reactions if exposed to Neem-Azal F, only at the lowest concentration of 1:50000 (approx.1 ppm Azadirachtin) few larvae survived. Decreasing numbers of larvae in the NP experiments during 48 hours were mainly caused by pupation and emerging of imagos, while any inhibition of pupation by Azidirachtin cannot not be withdrawn from these experiments. Larvae of phantom midges (Chaoboridae) survived all Neem-Azal F and Neem-Azal powder applications and pupated within observation time.

4. Recommendations

1. Further experiments are needed to test the adsorption/desorption processes of Neem Azal and stability of Azadirachtin under marine conditions. In 1992 the IfAH team will carry out laboratory tests with various salt water/ coral sand mixtures as well as with *Artemia salina* under varying salinity.

2. In summer 1992 laboratory tests with application of Neem Azal powder on larvae of European blood-sucking black fly species (*Wilhelmia*, *Simulium erythrocephalum*, *Simulium ornatum*) as well as on mosquito larvae (*Culex*, *Anopheles*) are planned by IfAH team.

3. We propose to start project phase 2, a three month pilot project in the Seychelles for test spraying of Neem - Azal instead of Diazinon on a 1 km beach section under controlled conditions. The investigations should include tests on the effect of so-called alternative methods (treatment of larval habitats with freshwater, sea water

and sand layers etc.) on the larval development of *Leptoconops spinosifrons* as well as the application of Neem-Azal powder. We recommend to apply Neem-Azal powder diluted into fresh- or seawater instead of Neem - Azal F which previously showed serious toxic effects on the terrestric and aquatic non - targed fauna.

4. Azadirachtin, the active substance of Neem-extract, is believed to affect insect development in two different ways: (a) The production of Ecdyson, the hormone that triggers pupation, is inhibited; (b) the development of ovaria in females is reduced. Provided that the development cycle is coupled tightly with lunar periodicity, tide levels and rainfalls, as is assumed in the technical literature, the application of Neem twice a month would be sufficient to reduce *Leptoconops* populations drastically. The first spraying would have to be performed 2 weeks before new moon (spring tide; inhibition of pupation of the last instar), the second about 1 week after new moon (reduction of ovarial development).

Acknowledgments

I wish to thank the following persons and organisations for support of the sandfly programme for sandfly control in the Seychelles:

- Ms.Inge Werner , IfAh Konstanz,and Dr.W.Joost (University of Leipzig) for their tireless help during our field investigations in the Seychelles
- Mr.Wolfgang Därr, Honorary Consul of Seychelles, for his continuous support
- the staff of Seychelles Tourist Office in Frankfurt and Mrs. T.Lablache, Seychelles Tourist Office on Praslin, for valuable help in the organisation
- The Ministry of Tourism and Transport, Rep. of Seychelles, for funding of the feasibility study and for permission to publish some of the results
- Air Seychelles for assisting in flight preparations and transportation
- Mrs. M.Chetty, Director General of Tourism Division, Ministry of Tourism and Transport, for supporting the project
- Mr.L.Chong Seng, Director of Tourism Support Services, Ministry of Tourism and Transport for his support and flawless organisation of our stay in the Seychelles as well as for valuable information on local conditions
- Mr.L.Bistoquet, MTT Praslin, for his tireless and efficient efforts to organize our investigations on Praslin and Curieuse
- Mr.N.Shah,Director of Conservation and National Park Division, Ministry of Environment, Planning and External Relations, Rep. of Seychelles, for permission to work on Curieuse Island
- Mr.A.Cedras, Park Ranger, for supporting our work on Curieuse Island
- Dr.H.Kleeberg, Trifolio GmbH Lanhnau, for free supply of Neem-Azal F and Neem-Azal powder and valuable informations and advices.

Literature

ABBOTT, R.T. & DANCE, S.P. (1990): Compendium of seashells.- (Melbourne, Florida) 4th edition, 411 pp.

DUVAL, J., RAJAONARIVELO, E. & RABENIRAINY, L. (1974):Ecologie de *Styloconops spinosifrons* (CARTER 1921) (Diptera, Ceratopogonidae) sur les plages de la côte Est

de Madagascar. Cah. ORSTOM , ser. Ent. med. et Parasitol. **XII**(4): 245-258.

ENGELHARDT, W. (1983): Was lebt in Tümpel, Bach und Weiher? - Kosmos Naturführer (Stuttgart), 10th edition, 257 pp.

FECHTER, R. & FALKNER , G. (1989): Weichtiere. - (München) , 287 pp.

LAURENCE,B.R. & P.L. MATHIAS (1972): The biology of *Leptoconops (Styloconops) spinosifrons* (CARTER) (Diptera, Ceratopogonidae) in the Seychelles Islands with descriptions of the immature stages. - J.Med.Ent. **9**: 51-59.

REYNOLDS, D.G. (1972): An aspect of medical entomology in the Seychelles Islands. - PANS **18**: 7576.

SCHRÖDER, P., I.WERNER & W.JOOST (1992): Control of sandflies (Ceratopogonidae) by non-chemical methods in the Seychelles. - Unpublished Final Report of a feasibility study in November 1991 to the Ministry of Tourism and Transport, Victoria, Republic of Seychelles, 43pp.

Naturally Occurring Substances of Plant Origin Used As Plant Protection Products: Authorization Requirements

E. Bode

Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig
Abteilung für Pflanzenschutzmittel und Anwendungstechnik
Fachgruppe Biologische Mittelprüfung

In general, people distinguish between two groups of agents for plant protection, the chemical and biological ones. This rather simple way of distinction is acceptable and convenient, not only for the public but, for instance, also for authorization of plant protection products in Germany and many other countries, including the future authorization according to the regulations of the European Communities (1).

This separation of active substances in chemical and biological substances (i.e. microorganisms including viruses) during the authorization procedure is justified because there are in general different approaches necessary for an appropriate examination and evaluation of "chemical" and "biological" products. Contrary to the often expressed opinion in the public, the reason for this procedure is not to look suspiciously at the "bad" chemicals and to look rather roughly at the "good" biological substances. It has, therefore, to be pointed to the fact that the way of examination for substances of each of these two groups will differ, but not necessarily the intensity.

Nevertheless, many people are regarding not only living organisms or, according to the definition given by FRANZ (2), parts of them as agents for biological control, but also include "biotechnical" or "biochemical" substances, be they extracted or synthesized, of microbial, plant or animal origin and exerting pesticidal or other biological effects (e. g. mating disruption, deterring from food, etc.).
There are several substances from plants known for long as "natural pesticides", e.g. nicotine, rotenone, pyrethrines, some of them being well known from authorization. During recent years, extracts from the neem tree (Azadirachta indica) have gained increasing importance in research for the development of plant protection products. The German authorities involved in the authorization of plant protection products are considering the extracts from the neem tree as chemicals as they are doing with all the plant substances mentioned above. This opinion is in accordance, for instance, with the following definitions given in the "Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC) (1):

Plant protection products: active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended, e. g. to protect plants or plant products against all harmful organisms or prevent the action of such organisms.

Active substances: substances or microorganisms including viruses, having general or specific action against harmful organisms or on plants, parts of plants or plant products.

Practice Oriented Results on Use and Production of ***Neem Ingredients;*** H. Kleeberg (ed.);

Substances: chemical elements and their compounds, as they occur naturally or by manufacture, including any impurity inevitably resulting from the manufacturing process.

In the German Plant Protection Act of 1986 (Gesetz zum Schutz der Kulturpflanzen - Pflanzenschutzgesetz, PflSchG; (3)) plant protection products are defined quite similar to the already mentioned definition of the Council Directive: substances which are, among other purposes, intended to protect plants against harmful organisms or non parasitic impairments or to protect plant products against harmful organisms. There is no German legal definition to characterize a substance, but chemical or biochemical substances comprising all substances, e. g. of plant origin, obviously will be classified as substances and during authorization procedure are examined and evaluated in the group of chemicals. As also pointed out, in the regulations of the Council Directive for authorization of plant protection products, only microorganisms including viruses are separated from chemical compounds (cf. "active substances"). This is the case, too, in the present German authorization procedure.

The German Plant Protection Act not only gives regulations for authorization of plant protection products but also for registering of plant resistance improvers ("Pflanzenstärkungsmittel"):
By definition, plant resistance improvers are substances which are intended solely to enhance the resistance of plants to harmful organisms (especially by way of "induced resistance") and which do not have a harmful effect on the health of man and animals or on the environment. This, of course, does not apply neither to neem extracts nor to other natural pesticides, e.g. pyrethrines or nicotine.

According to the present German regulations, usually plant protection products can only be marketed or imported if they are authorized by the Federal Biological Research Centre (Biologische Bundesanstalt für Land- und Forstwirtschaft). An authorization can be applied for by the producer, the marketing firm, if the plant protection product is to be marketed for the first time or by the importer.

The applicant has to submit an application form edited by the Federal Biological Research Centre. The data requirements set out in it are based on the Plant Protection Act and the Regulatory Ordinance on Plant Protection Products and Plant Protection Equipment (Verordnung über Pflanzenschutzmittel und Pflanzenschutzgeräte - Pflanzenschutzmittelverordnung; 4). Data to be submitted for the active ingredient (active substance) and/or the preparation cover

- chemical and physical properties of the preparation, the active ingredient(s), formulants and contaminants,
- analytical methods for the active ingredient(s), formulants and contaminants,
- the proof of efficacy against target organisms; quality of the crop (e.g. taste or odour, where appropriate); phytotoxicity,
- toxicilogy,
- residues and the fate and behaviour in the environment (soil, water, air; possible fate in food chains),
- ecotoxicology, e.g. effects on the activity of soil microflora, on soil fauna (especially earthworms), effects on aquatic organisms

(e.g. bacteria, algae, Daphnia sp., fish), on non-domestic mammals and birdlife, effects on honey bees and other beneficial organisms.

Many standard guidelines and leaflets exist, with precise details on how or even when to conduct the different trials necessary to fulfil the authorization requirements. It is known from experience that applicants do not always recognize which of the data requirements laid down in the application form they have to fulfil. Nevertheless, it is obviously impossible to give general instructions enabling an unexperienced applicant to find out all the reqirements he has to fulfil in his special case. Therefore, on request the authorization requirements for a preparation may be clarified between the applicant and experts of the competent authorities. This dialogue is very important and minimizes or even avoids confusions with the authorization procedure. Notwithstanding this fact the problem remains that often there are differring opinions as to whether a data requirement is justified or not, especially if the investigation is costly or if an applicant does not accept the need for a special reqirement because of the natural origin of the active substance.

The documents and samples necessary for demonstrating compliance with the authorization requirements are to be submitted together with the application form. If the examination of the data shows that

- the plant protection product is sufficiently effective in the light of scientific knowledge and technique,
- the plant protection product, when used for its intended purpose and in the correct manner, or as a result of such use,
  does not have any harmful effects on human and animal health or on groundwater and
  does not have any other effects, particularly with regard to the environment or the "natural balance" ("Naturhaushalt", soil, water, air, species of wildflora and -fauna, as well as the interactions between them), which are not justifiable in the light of the present state of scientific knowledge,

the authorization is to be granted by the Federal Biological Research Centre, acting in agreement with the Federal Health Office (where health is concerned) and the Federal Environmental Protection Agency (concerning prevention of damage as a result of water and air pollution or waste disposal). The authorization period usually is 10 years, but shorter periods can be provided. The authorization is renewable.

In the near future the authorization of plant protection products in the member states of the European Communities will take place according to the regulations laid down in the "Council Directive concerning the placing of plant protection products on the market (91/414/EEC; 1). Member states shall provide that a plant protection product may be authorized only if

- its active substances are listed in Annex I of this Directive ("Active substances authorized for incorporation in plant protection products"),
- it is sufficiently effective,
- it has no unacceptable effect on plants or plant products,
- it does not cause unnecessary suffering or pain to vertebrates to be controlled,
- it has no harmful effect on human or animal health, directly or indirectly (e.g. through drinking water, food or feed),
- it has no unacceptable influence on the environment, having

regard to
fate and distribution in the environment, particularly contamination of water including drinking water and groundwater,
impact on non-target species,
- the nature and quantity of its active substances and, where appropriate, any toxicologically significant impurities and co-formulants, can be determined by appropriate methods in general use,
- its residues, resulting from authorized uses, and which are of toxicological or environmental significance can be determined by appropriate methods in general use,
- if its physical and chemical properties have been determined and deemed acceptable for purposes of the appropriate use and storage of the product.

Authorizations shall be granted for 10 years and may be renewed. If need be, it will be possible to review authorizations at any time and in consequence to cancel them.

Applications for authorization of a plant protection product shall be made by or on behalf of the person responsible for first placing it on the market in a Member State to the competent authorities of each Member State where the plant protection product is intended to be placed on the market. The authorization in one Member State does not automatically imply the authorization in all other Member States, even if the authorization is granted on the basis of the regulations of this Council Directive. It is, on the contrary, necessary to make an application in those countries, too, where the use of the very plant protection product is intended. Following this application, the Member State must refrain from requiring the repetition of tests and analyses already carried out in connection with the authorization in another Member State. Of course, this regulation only applies if the agricultural, plant health, and environmental, including climatic conditions relevant to the use of the product, and the consequences of such use, are comparable in the regions concerned. Member States shall inform the competent EEC-Commission of cases where they have refused to authorize a plant protection product already authorized in a Member State. They shall, moreover, notify the Commission of the grounds on which repetition of a test was required or authorization was refused. To examine if a plant protection product fulfils the prerequisites for authorization listed above, the Council Directive 91/414/EEC comprises rather detailed catalogues of data requirements: Annex II includes the requirements for active substances, Annex III for the plant protection products. Both annexes are divided in parts A and B, where A stands for chemical substances (Annex II A) or chemical preparations (Annex III A) and B for micro-organisms and viruses (Annex II B) or preparations of micro-organisms and viruses (Annex III B).

However, certain pieces of information which would not be necessary owing to the nature of the substance or product or of its proposed uses need not to be supplied. In such cases or where it is not scientifically necessary, or technically impossible to supply information, a justification, acceptable to the competent authorities must be submitted by the applicant. With regard to the voluminous catalogues of data requirements, however, it is forseeable that applicants very often will not accept the

necessity of certain tests or investigations and in consequence will not supply all information the competent authorities want to get. In consequence, a dialogue between the applicant and experts of the competent authorities will be necessary as described already for the present German situation. Moreover, the problem remains that an applicant not always will accept that a "natural pesticide", e.g. with neem extracts or pyrethrines, has to fulfil similar authorization reqirements like other plant protection products with a "chemical" compound as active substance, especially when the investigations are costly. But one should always bear in mind that the natural origin of a substance does not necessarily indicate that its use as a pesticide does not affect human health or the environment. Examples are nicotine and the pyrethrines. Until recently, preparations with pyrethrines in the public opinion passed for excellent and "harmless" natural pesticides. Today, the application of those preparations is severely restricted or even banned by organic farmers!
Furthermore, decision making in the course of the authorization procedure has to be based on scientific knowledge and not on belief.

Literature

(1) Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC). Official Journal of the European Communities No. L 230.

(2) Franz, J. M. und A. Krieg, 1982. Biologische Schädlingsbekämpfung. P. Parey, Berlin und Hamburg.

(3) Gesetz zum Schutz der Kulturpflanzen (Pflanzenschutzgesetz - PflSchG) vom 15. September 1986. BGBl. I, 1505 ff.

(4) Verordnung über Pflanzenschutzmittel und Pflanzenschutzgeräte (Pflanzenschutzmittelverordnung) vom 28. Juli 1987. BGBl. I, 1754.