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2015 Expo. The Neem Cake Project. From By-Product of Industrial Process to Multipurpose Resource for a Sustainable Agriculture Chain

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Abstract

The 2015 EXPO (International Exposition, Milan, Italy, May-October 2015) was dedicated to the theme: Feeding the Planet, Energy for Life. The EXPO hosted an International Call on "Best Sustainable Development Practices for Food Security". The project "Neem Cake: for a Sustainable Agriculture Chain, the Multipurpose By-Product of an Industrial Process" gained a prize as one of the best among the participating projects, in consideration of the innovation and possible results. Main elements of the project, as well as its conceptual foundations, are here presented. The project was an occasion to focus on the utilization of neem cake, as an example of application of new plant derived products in agriculture and food production.

Keywords: *Neem cake; Azadirachta indica; Agriculture; Food production*

Introduction

2015 EXPO (International Exposition, Milan, Italy, May-October 2015) was an important occasion to debate about food and nutrition. Several solutions and projects were presented. Here, information is reported about the project "Neem Cake: for a Sustainable Agriculture Chain, the Multipurpose By-Product of an Industrial Process". The project was an occasion to focus on the utilization of neem cake, as an example of application of new plant derived products in contrast with actual dominant paradigms in agriculture and food production. The project, in consideration of the innovation and possible results, gained a prize in the International Call on "Best Sustainable Development Practices for Food Security". Main elements of the project, as well as its conceptual foundations, are here presented.

The Occasion

Expo 2015 was a Universal Exposition hosted by Milan, Italy. The opening took place on May 1, 2015 and the Expo closed on October 31, 2015. In an area extending over one million square meters, the EXPO received more than 21 million visitors and the presence of 145 countries, several of them presenting in a dedicated space their products and projects. The theme chosen for the EXPO 2015 was *Feeding the Planet, Energy for Life*. It embraced technology, innovation, culture, traditions and creativity and how they relate to food and diet. This common thread runs through all the events organized both, within and outside the official Exhibition Site. Italy was the ideal site for the event, being food and nutrition at the top of interest in this country. Each town, each village, is proud of its own tradition with local dishes and wines, as the result of a millenary cultural heritage. The quality and variety of the Italian food and related products is well worldwide known.

Expo 2015 provided an opportunity to reflect upon, and seek solutions to, the contradictions of our world, evidenced by the distribution of food supplies. On the one hand, there are still the hungry (approximately 870 million people were undernourished in the period 2010-2012) and, on the other, there are those who die from ailments linked to poor nutrition or too much food (approximately 2.8 million

deaths from diseases related to obesity or to being overweight in the same period). In addition, about 1.3 billion tons of foods are wasted every year. Another evidenced argument was related to the quality of food and the main consequence concerning the Obesity. Obesity is essentially an abnormal or excessive fat accumulation. Alert is connected with consequences on health, since obesity is reported as the second leading environmental cause of death in USA and the fifth of global deaths. Obesity is considered an epidemic phenomenon, interesting many parts of the world, and therefore it was named by the WHO with the suggestive term of Globesity. Everywhere, the obesity phenomenon is now of primary importance, interesting directly the food production.

The Expo theme becomes a unique occasion of sharing and celebration, involving ordinary people and leaders in conferences, events and meetings. It was stressed the need to make conscious political choices, develop sustainable lifestyles, and use the best technology to create a balance between the availability and the consumption of resources.

In occasion of the 2015 EXPO, a team of public and private subjects presented a project based on neem application, replaying to the International Call on "Best Sustainable Development Practices for Food Security". The project, named "Neem Cake: for a Sustainable Agriculture Chain the Multipurpose By-Product of an Industrial Process", was selected at the top level in the Expo Milano 2015 among the 71 presented.

The Background

Probably, one of the main conclusion of EXPO is that agriculture is going to face in XXI century fundamental challenges that can be overcome only by innovative solutions. The complex environmental sap producing the challenges is generated by changes in act. Climate changes generate desertification and massive migrations of insects and humans [1]. The resistance phenomenon increases, interesting antibiotics and insecticide and involving the definitive decline of synthetic products in favor of the natural ones. The resistance could give rise to the appearance of genetic chances, generating more virulent and aggressive variants of the present ones.

The next future of agriculture will be deeply influenced, with damages in quantitative and qualitative aspects of food production. These novelties cannot be faced with the current arsenal. Besides the new antibiotics and biocidals, we need innovative ideas, against the actual dominant paradigms, that are the main responsible of this situation. The Project is the result of the effort in concretizing the novel paradigms in agriculture and food production, mainly based on natural products and recent technological advances.

The Neem Tree

The neem tree, *Azadirachta indica* A. Juss (Meliaceae), is a fast growing evergreen tree native of Indian subcontinent, where it is largely present and considered a high value medicinal plant, utilized on several therapeutic treatments. Neem was valued as a potential important tree for human health and environment care [2,3]. The main product of neem is the oil obtained by cold expressing the kernels containing the seeds, mainly used as insecticide [4]. Emulsified formulations of A. *indica* oil showed an excellent larvicidal potential against different mosquito genera, including *Aedes, Anopheles* and *Culex*, also under field conditions. Many formulations deriving from neem seeds show anti feedancy, fecundity suppression, ovicidal and larvicidal activity, insect growth regulation and/or repellence against insect pests, even at low dosages [5-11]. Other advantages arising from the use of neem-based products are the rare induction of resistance, due to their multiple mode of action against pests and the low toxicity rates that have been detected against vertebrates, and environmental care. Security in use, environmental care and absence of toxicity for non-target organisms and humans were testified by US EPA [12].

The Target

Serious limitations in the use of neem oil as insecticide derives by the relatively high cost of refined products and the low persistence on treated surfaces exposed to sunlight [13]. Furthermore, although about neem attention and economic relevance is actually focused on the oil, neem potentiality is far to be explored, and other parts of the plant as well other products deserve consideration, like neem cake.

Nowadays, neem cake is an industrial low-cost by-product, consisting in the residue after neem oil production. However, it consists in a heterogeneous material that maintains a high added value, due in large part to its chemical composition that confers its biological activity. The neem cake is widely available on the global market, including the Italian market, but production should increase rapidly in the next years, due the increase of neem cultivations. Recently, neem changed completely its distribution, expanding by massive cultivations in sub-tropical regions of America (Caribbean Cuba, Central and Southern America), Asia (Nepal, Pakistan, Bangladesh, Sri Lanka, Myanmar, Thailand, Malaysia, Indonesia and Iran, China, Turkey, Indonesia), Africa (Cameroon), even in Queensland in Australia no less them half a million of the tree has been planted recently. The reason of neem expansion is due to its utility in fighting desertification [14]. Neem is tolerant to most soil types, including dry, stony, lateritic crusts, highly leached sands and clays, including marginal and leached soils.

The potentiality of neem cake as functional fertilizer is founded on two dual opposite effects on ground biotic composition: promote the development of useful microbe biomass and the control of other ones as an insecticide and nematicide. Neem cake has a different chemical composition from the oil and its properties indicate its possible use on a large scale, consistent with the fact that on the world market there are huge amounts of the product. The withdrawal from the market of polluting agrochemicals makes very interesting neem cake exploitation.

The project is focused on the exploitation of neem cake characteristics to obtain the following targets: a) impact in the food chain to improve the consumer healthy; b) increase of the productivity of agriculture products; c) to feed the planet as the logical consequence of the urgency to develop new sustainable agricultural systems in a world where many highly polluting pesticides have, no longer, permission to be used.

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The project consists in an attempt to know the real value of its microbiological, insecticide, fertilizer, nematocide activity in field conditions [15,16].

The Partnership

The project is the result of collaboration among different individuals: import companies, organic farms and research institutions, in order to determine the manner and timing of land application of neem cake, as valuable product of 'waste', still underestimated. Each counterpart has specific competences, to obtain a successful integration inside the project.

ENEA (Ente Nazionale per l'Energia e l'Ambiente)

ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development) is one of the largest scientific and technological state-owned Italian Research Institution. Nowadays, ENEA's main aims are connected with the sustainable use of natural sources, like to promote innovation technology activities, to disseminate and transfer technologies encouraging their use in production and social sectors; to provide high-tech services to public and private bodies and enterprises. In the project, the targets of ENEA's participation are related to the management of crop resources, by conduction of field experiments trials, innovation in the agro-industrial production system for competitive and sustainable food products, exploitation of new raw materials and foodstuff in the area of functional foods.

Department of Agricultural Sources, University of Bologna, Italy

Activity of the Department is focused on fruit and forest trees as well as in viticulture, ornamental species and landscape management. Research deals with biology, eco-physiology and genetic in order to improve the knowledge on plant metabolism, nutrient cycles, breeding and fruits quality, into the development of sustainable agriculture, environmental preservation and varietal innovation.

Plant Pathology and Entomology Section of the Department of Agriculture of the University of Sassari (Italy)

It is a research institution working on several applied entomology subjects. Main targets are the integrated pest managements to protect Mediterranean crops, the study of medical veterinary parasites and the Apidology. Expertise are the monitoring of insect populations, sampling techniques, evaluation of pesticide efficacy in the field and impact on non-target arthropods.

FAO (Food and Agricultural Organization of the United Nations)

Achieving food security for all is at the heart of FAO's efforts – to make sure people have regular access to enough high-quality food to lead active, healthy lives. FAO's three main goals are: the eradication of hunger, food insecurity and malnutrition; the elimination of poverty and the driving forward of economic and social progress for all; and, the sustainable management and utilization of natural resources, including land, water, air, climate and genetic resources for the benefit of present and future generations.

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The laboratories of the section of Pharmaceutical Botany at the Department are specialized in the control of quality of complex plant extracts using the fingerprint metabolome approach. The metabolic production, in particular of secondary metabolites, needs a complete analysis evidencing, as possible, the total presence of constituents. This is important when the activity cannot be attributed to single substances, like evidenced in the analyses. Important results were obtained using the HPTLC (High Performance Thin Layer Chromatography) adapted to botanical food supplements and plant extracts.

Farms and experimental fields

The farm of di Montecorvino, Pugliano (Sa, Italy) and several experimental fields were utilized to test neem cake effects of several species, from herbs to trees. In all cases, neem cakes increased leaf chlorophyll content and had a positive effect on plant growth.

The Chemical Part of the Project

One of the aims of the Project was the necessity of obtaining a reliable, valid and low cost analytic method for control quality of neem products. The neem seeds contain at least one hundred biologically active compounds. More than one-third of them are tetrahydron or triterpenoids, with azadirachtin A, salannin and nimbin as the main constituents [17]. In many cases, partial loss of the lateral chain is combined by a complicated rearranging of the remaining part, giving rise to different polycyclic molecular skeletons, full of oxygenated functional groups, partially acylated. Most of neem seed oil is produced in India by familiar little producers, but many other countries, like Thailand, are now producing the oil with different methods. Therefore, considering also the possible different geographical origin of the raw material, combined with pre- and post-harvesting factors can result in great differences in constituents present in marketed neem oils.

The expressed kernel is the resulting component of neem cake, after the production of the fixed oil. The neem cake is a power with different colour and smell, according to the expression method. The procedure can be repeated, obtaining the deoiled neem cake. Neem cake is a vegetable matrix with a variable chemical composition regarding the content in the secondary metabolites typical of neem tree. HPTLC (High Performance Thin Layer Chromatography) was selected as useful tool for control quality of neem products. HPTLC is the last evolution of planar chromatography [18]. Allowing to the capacity in evidencing natural products, including also those in very low concentrations, HPTLC is used to perform metabolome studies, like determination of most of the constituents of an extract [9,10,19-25]. Main product of HPTLC analysis is the chromatographic fingerprint, consisting in the individual track typical of the extract

or the product. Chromatographic fingerprint analytic approach received important official recognitions. Plates can be visualized and derivatised in several ways, obtaining multiple information, as well as converted in a series of peaks by densitometric treatment. In such way, the comparison between samples is reliable and facilitated by the visual inspection and samples can be analysed side-by-side and exactly in the same conditions [18].

HPTLC analysis of extracts of different commercial samples of neem cakes (Figure 1-3) showed a complex composition, being the analysis able to show a wide range of different products, characterized by the Rf value and differently evidenced. The total MeOH extracts (Tracks 1-3) were compared with the EtOAc extracts (Tracks 4-6), and both compared with selected limonoid standards (Tracks 7-9). As expected the tracks of extracts in the same solvent are similar confirming the utilization of the same raw material, but also several differences; mainly quantitative are present. The red spots differences, corresponding to the fatty acids and triglycerides, can be easily are different mainly because of the used extraction methods in the oil production. Other significant differences, including the limonoid spots can also assigned to the production method of the oil, but also provenience is important, as well method of cultivation and storage, since they also observed in the neem oil samples. These content differences are actually important in the properties and efficacy of the neem products, asking for a careful quality control that, as evidenced, can be efficiently obtained by the HPTLC analysis.

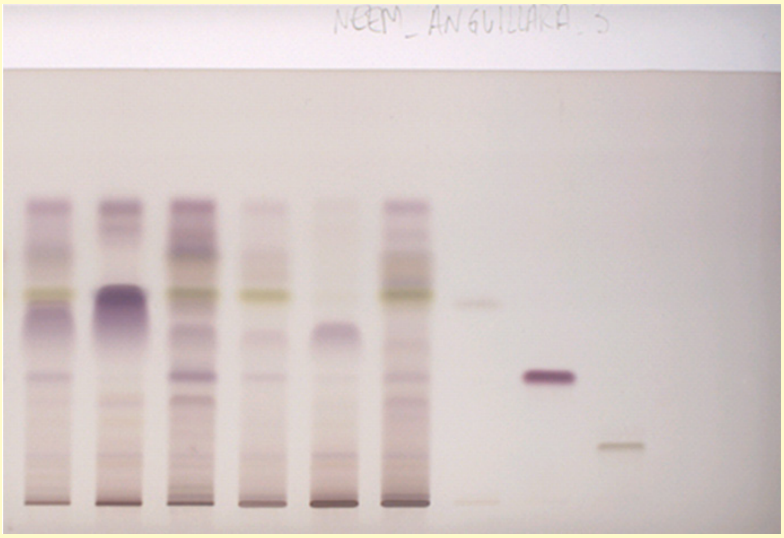


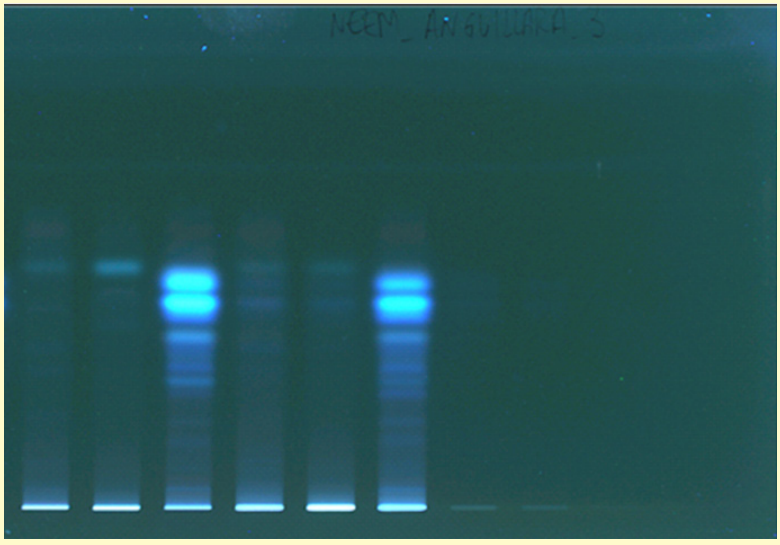
Figure 1: *HPTLC analysis of different commercial samples of neem cakes. Mobile phase: Toluene/ethylacetate 4:6 (v/v). Derivatization: Absent. Revelation: upper and lower white light. Tracks: 1-3, AcOEt extracts; 4-6, MeOH extracts; 7, Nimbin; 8, Salannin; 9, Azadirachtin A.*

Experimental

HPTLC analysis

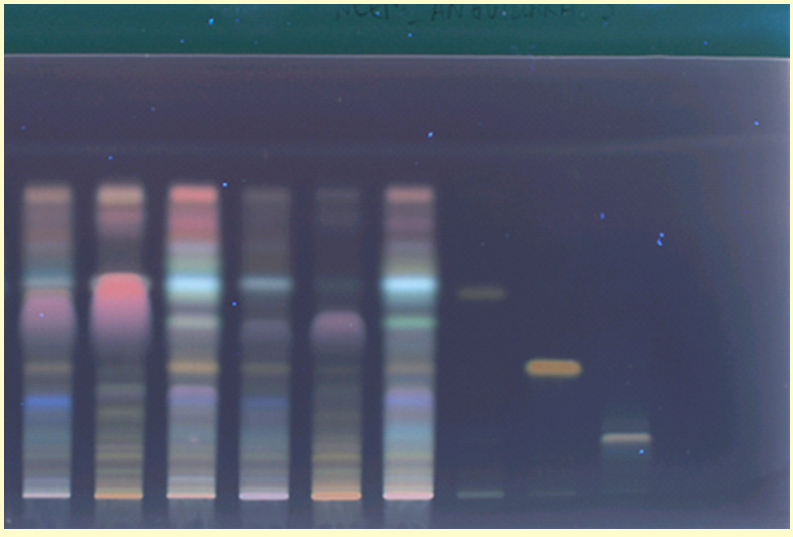
Material

Analysed samples were obtained from the market (information about the commercializing firms are available under request).



1 2 3 4 5 6 7 8 9

Figure 2: *The same plate of Figure 1 evidenced at 366 nm.*



1 2 3 4 5 6 7 8 9

Figure 3: *The same plate of Fig. 1 evidenced at 366 nm, after derivatization with any aldehyde.*

HPTLC Device

The HPTLC system (CAMAG, Muttenz, Switzerland) consisted of Linomat 5 sample applicator using 100 µl syringes and connected to a nitrogen tank; chamber ADC 2 containing twin trough chamber 20 x 10cm; Immersion device III; TLC Plate Heater III; TLC visualizer linked to winCATS software. Glass plates 20cm x 10cm (Merck, Darmstadt, Germany) with glass-backed layers silica gel 60 (2 µm thickness). Before use, plates were prewashed with methanol and dried for 3 minutes at 100°C. Filtered solutions of extract and

standards were applied with nitrogen flow. The operating conditions were: syringe delivery speed, 100 nl s^{-1}; injection volume, 4 µl; band length, 8mm; distance from bottom, 70mm. The HPTLC plates were developed in ethyl acetate : dichloromethane : acetic acid : formic acid : water (100 : 25 : 10 : 10 : 11; v/v/v/v) using the automatic and reproducibly developing chamber ADC 2, saturated with the same mobile phase for 20 minutes at 25°C. The developing solvents (i.e. type of solvents and ratios) were carefully optimised before the analyses. The length of the chromatogram run was 70mm from the point of application [21,25].

Analysis

The developed layers were allowed to dry at 100°C for 5 minutes and then derivatised with a selected solution, including Natural Product Reagent (NPR) (1g diphenylborinic acid aminoethylester in 200 ml of ethyl acetate), the plate is heated at 100°C for 2-3 minutes and then dipped into anisaldehyde-sulphuric acid (1 ml p-anisaldehyde, 10 ml H_2SO_4, 20 ml AcOH in 170 ml MeOH). Finally, the plates are dried for 5 minutes at 120°C before inspection. All treated plates were then inspected under a UV light at 254 or 366 nm or under reflectance and transmission white light (WRT), respectively, at a Camag TLC visualizer, before and after derivatisation. WinCATS software 1.4.4 was used for the documentation of derivatised plates [21,25].

Validation

Sample solutions of the extracts were found to be stable at 4°C for at least 1 month and for at least 3 days on the HPTLC plates. Repeatability was determined by running a minimum of three analyses. RF values for main selected compounds varied ± 0.02 %. The effects of small changes in the mobile phase composition, mobile phase volume, duration of saturation were minute and reduced by the direct comparison. On the contrary, the results were critically dependent on prewashing of HPTLC plates with methanol [19,21].

Results and Discussion

Other Experimental Evidences

Good Practice of Cultivation: The target was the cultivation of watermelon, Minirossa (Lamboseeds), yield 65 t/ha, fertilization and nematodes control only with neem cake. Root system was practically undamaged from root-knot nematodes, against the ground, in pre-transplant, with 15 q.li/ha in a soil strongly infested.

Culicoides and sheep: *Culicoides* are small blood-sucking Diptera belonging to the family of Ceratopogonidae. They play an important role in the transmission of several vector-borne pathogens such as Bluetongue virus which affect sheep. *C. imicola* is the main vector of Bluetongue Virus in Africa and Mediterranean Basin. In the last 14 years more than 600.000 sheep have died of Bluetongue Disease in Sardinia. The tests showed the efficacy of neem cake in control of Bluetongue vectors in field conditions. Field trials carried out in Sardinia revealed that neem cake controls better than the other larvicidae Culicoides larvae on breeding sites, and its activity last for a long period of time, around a month [26-28].

Fertilization in kiwifruit orchard: Soil application of neem cake was tested in a mature commercial orchard (cv Hayward), in river Po valley. Lime induced Fe-chlorosis represents one of the most important nutritional disorder of susceptible crops when grown on alkaline-calcareous soil. Excess of nitrate-nitrogen in soil has been indicated as one of the possible cause of iron deficiency and soil-applied neem cake has been proposed as a sustainable strategy to reduce the nitrification rate, thus improving Fe-availability for plant uptake.

Neem cake as fertilizer: A farm of di Montecorvino, Pugliano (Sa) began a trial: 1000m of land, cultivated at arugula, fertilizer with 1q of neem cake. Results after a month showed absolute effectiveness of fertilization and pest management method in greenhouse conditions. For next sowing in February, the farm planted 2ha of arugula (*Eruca sativa)* in greenhouse treated with 20 q of neem cake. To sell product on the organic market of fresh-cut salads to a foreign buyer, required chemical analysis of pesticides and heavy metals of

used neem cake were performed. Results confirmed the appropriateness of use of neem cake according to organic protocols applied for salad IV gamma.

Project Innovations

In contrast with reported information, the results of recent researches showed that the efficacy of neem cake is not depend on the content of azadirachtin. The results of the chemical analyze for the content in neem pesticides and heavy metals, thereby showing that fertilization with neem cake meet the requirements for the marketing of organic production. These analytical data establish a standard of quality for the exploitation of the product. The results described in our applications have stimulated synergies with researchers and companies that deal with other areas of application.

The neem cake led to a revolutionary improvement in the fertilization of agricultural plants, adding to the characteristics of chemical fertilizer those of soil improver. We could define the neem cake a prompt nutrient-release fertilizer, effective in allowing rapid absorption of nutrients and promote development of the plant and increase the activity of the microbial biomass and organic matter, favoring the sequestration of carbon.

The exploitation of the use of neem cake as insecticide came from this first test: some pots of impatiens plants were fertilized with 3% by volume of neem cake, 500 mosquito larvae were reared starting from the eggs. The eggs hatched in control and treated pot saucers, but none of the new borne larvae survived in the saucers water of pots treated with neem cake, while in the water saucers of pots unfertilized with cake the 500 control larvae completed in less than a week their development becoming adult mosquitoes.

The main innovation of the Project lies in the proposal of using neem cake in various sectors of the food chain, demonstrating the extraordinary potential of this industrial by-product, to increase the quality, quantity and safety of food production. The neem cake is a natural eco-friendly, sustainable, low cost, easy to produce product, able to introduce improvements in agrarian ecosystem. Improvements are concentrated into: 1) in availability of nutrients, more consistent with the requirements of the crop; 2) in development of the microbial biomass, which increases in quantity and activity; 3) in development of agricultural plants, which in addition to nutrients can count on a greater variety of useful microorganism and on acquisition of nutrients themselves, through the activation of complex symbiotic systems; 4) in development of new pest control system, regarding of soil nematodes and insects of agricultural and livestock interest [28].

Final Proposals

Finally, it is necessary to recall some of the main beneficial effects of neem cake utilization.

Beneficiaries are farmers in pest and nematode control. In particular for nematodes; currently, highly toxic products can still be present on the market by virtue of a 'extraordinary authorization' by the European Commission, but the complete ban of this pesticide would leave farmers without conventional alternatives. Furthermore, farmers could count of new revolutionary product for plant fertilization. Other major beneficiaries of the use of neem cake as insecticide are undoubtedly sheep farmers who can use an organic product of natural origin and low cost that is simultaneously effective against the larvae of Culicoides and respectful of the natural biotic communities.

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