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Current mosquito-borne disease emergencies in Italy and climate changes. The neem opportunity

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Abstract
General concern is that global climate changes will be very important for mosquito-borne diseases diffusion. Three examples concerning current alarms in Italy are reported. The three examples concern different vectors and different stories, from novel introduction of aggressive and dangerous species (Tiger mosquito) with general damages in the country to new virulences of already known diseases (*Xilella fastidiosa*) and BTV (Blue Tong Virus) in particular regions (Puglia and Sardinia, respectively) Microorganisms have a great advantage to adapt easily to environment, being able to change their genome more rapidly than our genome with a mutation rate of approximately 0.3% per million years. Our genetic background is not able to keep up this increasing mismatch. Therefore, as already experienced, technology is our only possibility to perform tools to face changes and their consequences. Any real utilization for any new need or necessity is a challenge of interdisciplinarity researches. After the disasters derived by the use of synthetic insecticides, natural products seem in pool position for developing of a new generation of eco-friendly and sustainable control of borne-insect vector diseases. The current studies for controlling insect vectors at the larval stage using natural products from neem tree (Azadirachta indica) are reported.

Keywords: Neem cake, *azadirachta indica*, plant animal insect vectors, bluetongue virus, tiger mosquito, *Xilella fastidiosa*, fastidious prokaryotes

Introduction
I will send swarms of flies on you and your officials, on your people and into your houses. The houses of the Egyptians will be full of flies; even the ground will be covered with them.

Bible, Exodus 8, 16.

Consciousness of the consequences of global warning is still worldwide very low and inadequate. Actually ordinary people consider climate changes as about how much rain or snow they have to face or how hot will be the next summer. People are not aware of the impact on other aspects of life including food and health. In any case people feel powerless and most of mankind is living in Asiatic countries where industrialization is achieved at any cost, as the only possibility of improving lifestyles. Absolutely without any aware of the future changes are the governments, despite all warming from the scientific side. So far, despite the evidences in human activities influences scientist's main role was confined in recording the effects of the climate changes in weather conditions without any possibility to be protagonists. Perhaps the approach and the method used to inform the public are wrong. Attention should be focused not only on the planet climate changes, but on the secondary consequences on the ordinary life, like those derived from the insect migration and its consequences in the environment that we love and where we use to live. The new warming could led to a preoccupation about effects on ordinary lifestyles and consciousness for investing money, intelligence and diligent effort in developing strategies of control and novel tools adequate to current challenges. The general message about the current climate change is that the temperature of the planet is increasing, probably as a consequence of human activities impact [1-3]. However, our planet is a complex dynamic system. The recorded increasing temperature is the sum of singular situations, that must be considered singularly, to understand what is going on. The term change must be converted in changes. Climate evolution in Europe is an interesting example to understand the current situation [2,4].

In Europe several marked changes in climatic conditions occurred during the last 1000 years, when two main warming periods has occurred, separated by a colder period, named Little Ice Age. The first period is called Medieval Warm Period and it is located in 950-1500 a.C., corresponding to warmer areas in certain regions [5]. The Medieval Warm Period had

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many effects on European history, influencing deeply human activities in the Renaissance, mainly concentrated in Italian peninsula. It caused significant changes in trading, medieval farming and husbandry. The English farmers were able to compete with the French in producing high quality wine. It was also a period of strong contrasts. Climate change shaped the nutritional environment by creating crop surpluses but also famines affecting in general the health of the medieval population, determining changes in diet and nutrition. Other historic characters were the explosion of creativity in arts and explorations. However the climate change also contributed to the spread of many diseases including the famous bubonic plague. The impact of these pests on mankind are depicted in universally known literature masterpieces, from Dante to Shakespeare, from Boccaccio to Manzoni.

The second warm period is in act, with the warmest decade (the 1976-2000) on record and with many areas involved. The evidence was an increasing of approximately 1.2°C over the past 100 years, that means twice the expected average global rate [6].

Warning general consequences in interested areas are higher nigh-temperature, with limited difference between day and night, and few frost days in winter, associated with milder temperature in all winter period, longer dry periods and peaks of temperature. In particular, temperature increases were most evident in both South Europe (Italy, Corsica and Balearic Islands) and Eastern Europe (western Bulgaria, northern Greek, Albania, Macedonia, Bosnia, Montenegro, Croatia). On the contrary central Iberia, and the region around the border between Morocco and Algeria have cooled. Simplifying the model tendency; South Europe is getting warm and North Africa is cooling.

The above data concerning the temperatures can be connected with those regarding precipitation. The final result divides Europe in two parts, according to the temperature: the number of wet days have increased in the North Europe and decreased on the South. North Europe, including UK, northern Iberia and Scandinavia are becoming wetter, whereas southern Iberia, France, Germany and Italy are becoming drier.

Thus the global warming refers to sum of the climate changes in the planet, that means the sum of microclimates data. However this means that each situation can experience great differences, including a high warming for some parts of the continent and the contrary for others. This is the key to understand several effects, including the migration of the insects [6,7].

Bacteria, as other microorganisms, are too little to spread efficiently in our ordinary habitat. Most of them have developed certain alliances with insects. These bacteria loose their independence but become not only able to move in the space but also in the environment, being in condition to utilize different hosts. The acquired capacity is unique in the living organisms and represents a sure advantage in competition for surviving. There are consequences for us, when we become the host target: the most important human pests–malaria, yellow fever and dengue–are mosquito-borne. Their histories must be examined in the contest of past climates and the other factors that can influence their transmission and actualized the effects of the last environmental changes.

Vector-borne pathogens are particularly sensitive to climate [6,7], and in particular case insects. A widespread and continued speculation concerns the incidence and intensity of their transmission by anthropogenic climate change, although other non-climatic abiotic and biotic factors can also affect disease distribution, as well as cooperate to the phenomenon. Human activities impact consideration was so far focused mainly in effect of pollution (acid rain, ozone, etc.), but the situation is more complex and clime should be better considered, including all aspects of the environment. We are now going to examine some cases occurring in Italy and concerning actual vector-borne insect plagues and the experiments in act to control or limit their diffusion. Insect diffusion can be very rapid and effective. Adult insects are usually not strong fliers but they can be passively dispersed by the wind, possibly up a hundred kilometres in a single night, especially over the sea. Otherwise they can travel utilizing ancient transportations, like other animals (street ruminants), or new unexpected ones, i.e., inside old tires. Therefore rapid diffusion is not a problem if climate conditions are favourable to survive and entrench. For these reasons countries so far considered safe, as Central and North Europe, are now interested by menace of new invaders and the alarm is currently extended in all Europe. As well know from antiquity, insect reproduction is high sensitive and strictly linked to clime conditions, causing absence of the insects in one year and biblical plague in the next season. Often the change of habitat can be very favourable for the insect and its pathogen. They leave usually in a situation in equilibrium, where the natural enemies and limiting resources determine the control. Climatic conditions are determinant for diffusion, if they change in favour, the plague mechanism is acting. The traditional controlling factors are absent; and in few years the autochthonous competitors species are eradicated and the new habitats assimilated. Effective methods of control usually are absent owing the novelty.

We are presenting three cases of current emergencies in Italy, focusing on the different characters (**Table 1**). The expanding of Tiger mosquito [8,9] is a classic example of this smooth operation. Another situation concerns pathogens, that stated silent for long time until a mutation generated a different virulence with consequence enhanced by climate change. Usually the consequence of the genetic upgrade is even more devastating, as reported later, for *Xilella* [10,11]. The third case concerns pathogens that are strictly linked to a selected host, like the bluetongue story [12-14].

Beside the analyses scientists should find solutions. There is an urgent need for ecologically sound, equitable and ethical pest management, based on control agents that are pest-specific,

Table 1. Current mosquito-borne disease emergencies in Italy.

Vector	Disease common name	Pathogen	Occurrence in Italy
Aedes aldopictus	Asian tiger mosquito	*Alphavirus chikungunya*	Large diffusion in all the Country
Culicoides imicola	BTV (Blue Tongue Virus)	Arbovirus	Sardinia region
Hemiptera–aphids, leafhoppers, white flies, mealybugs	--	*Xilella fastidiosa*	Puglia region

nontoxic to humans and other biota, biodegradable, less prone to pest resistance and resurgence, and, last but not least, relatively not expensive. The last aspect is fundamental for a large scale use in emerging countries. In the last decades several strategies for insect control were undertaken [15]. Among them, biotechnological approaches based on genetically modified organism [16,17]. The transgenic approach to arthropod control foreseen the germ-line transformation of host arthropod genome to obtain male insect vectors refractory to pathogens [18-20]. Other approaches pursue mass-rearing and field release of sterile male insects [21-23]. However, since no legal framework exists to authorize the release of such organisms in the nature, sterilization by irradiation remains the most used technique [24-26].

Most pathogens vectored by arthropods, such as *Plasmodium* and dengue virus, must undergo an extrinsic incubation period (EIP) in the vector, before they can be transmitted to a new host. This developmental period for the pathogen often comprises a significant proportion of the expected lifespan of the vector [27]. As such, only a small proportion of the oldest population contributes to pathogen transmission. Given this, strategies that target vector age are under investigation to obtain the most significant reductions in the capacity of a vector population to transmit disease. But, given the rapid and widespread evolution of insecticide resistance, it would seem prudent to consider potential selection pressures that any of these life-shortening strategies may impose on both the pathogen and vector populations. One concern is the possible development of resistance to these life-shortening agents by the insect vectors.

Actually attention shifted on natural products, considering the difficulties in going on with synthetic pesticides. Among various options concerning botanical bio-pesticides, we identified neem as a source of eco-friendly "soft" natural pesticides. We focused on larvicidal activity of neem products, in particular neem cake, in order to obtain the scientific background for the developing of a new, effective, safe, sustainable, low cost and easy to use insecticide, to be utilized also in domestic situations [28].

The insecticidal activities of neem

Several impressive words have been spent to qualify Neem (*Azadiracthta indica* A. Juss), such as : the "marvellous tree", "the solution tree" "the village pharmacy", the "botanical marvel". Neem, identified by WHO/UNEP1989 as an environmentally powerful natural pesticides, is considered to be one of the most promising trees of the 21st century for its great potential in pest management, environment protection and medicine [29]. The genus *Azadirachta*, member of the Meliaceae (Mahogany family), consists of few species, wherein *A. indica* is by far the most important one for diffusion and applications. Usually it is a beautiful hardy tree, reaching also monumental features. Neem is indigenous to the Indo-Pak subcontinent (Pakistan, India, Bangladesh, Myanamar), however owing its ability of growing easily and rapidly (80 cm in one year) it is now globally cultivated in many tropical and subtropical countries, including South Asia, West Africa, Central (Cuba) and South America, Australia, the Plains of Arafat (Turkey). Actually it was introduced and heavily cultivated also in China. From January to May abundant fragrant and beautiful flowers are produced, and fruits ripen June through August, as green ellipsoidal drupes containing one seed. A single mature tree may produce annually 5-8 Kg of seeds.

India is the original country of neem, and still remains the worldwide leader producer. Commercial uses are actually restricted to removable materials, such as leaves, fruits and mainly seeds. There are about 14 million neem trees growing only in India meaning a potential production of 3.5 million tonnes of seed/year, corresponding to a production of 700,000 tonnes of oil/year. Currently, in the market there is a huge amount of products obtained from this multipurpose tree, due to the anti-fungal, anti-bacterial and dermatological properties, but the most commercially important product is the neem oil extracted from its seeds, also known as margose oil [30].

Use of neem oil formulations on sucking and chewing pests provides a greater degree of pest control, as it affects their digestive system. Botanical insecticides, extracted from neem oil, contain probably more that 400 constituents, including several complex tetranor-triterpenoid, named limonoids, considered the responsible for both antifeedant and toxic effect in insects. They are azadirachtin A, the predominant one in neem oil, azadirachtin B (3-tigloyl-azadiracthin), nimbin, salannin and related compounds, that have proven to be effective in the control of agricultural pests. There is a huge scientific literature, which reveals both the antifeedant and the physiological effects of these substances. The different formulations of neem expeller oil have a content of azadirachtin from 300 to 2000 ppm. The oil is obtained usually by large mechanical expellers or by solvent extraction, only small-scale producers still use traditional pressing methods, that however are widely spread especially in India. There are two main different neem cakes in the market. Neem oil cake is the by-product obtained in the cold-pressed process with an oil content of about 6%, the neem de-oiled cake, the by-product obtained in the organic solvent extraction process, that has still an oil content of about 1.5%. However other types of marketed neem cakes are present according to the

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used extraction method. Both in the neem oil and in the neem cake commercial products the chemical composition resulted very different according to the provenience and the production process.

The only present utilization of neem cake concerns its use as a natural and environmental friendly fertilizer, soil conditioner, nitrogen saver and manure in farming and agriculture. We decided to explore other possible uses of neem cake. We focused our study in the development of a new domestic insecticide using neem cake as raw material, to be used against biting flying insects present in urban areas. Neem cake was selected on the basis of low cost, sustainability, availability of enormous quantities, current uses in agriculture and possible direct utilization. However, the validation of neem cake as insecticide is still to be obtained.

Emergency in sardinia: The (BTV)

BTV (Bluetongue virus) is a devastating disease of ruminants, due to a type species of the genus *Orbivirus* [31]. The virus replicates in all ruminants species, but severe diseases are mostly restricted to selected breeds of sheep, that are high appreciated for fine wool and common in certain countries of Europe. Since BTV arrived in Europe in 1998, either by the movement of infected ruminants or by the wind-dispersal of infected midges, six strains of BTV have spread, interesting 12 countries and 800 Km, causing the deaths of more than one million of sheep [32]. BTV is transmitted between its ruminant hosts almost entirely by the bites of certain species of *Culicoides* midges (Diptera: Ceratopogonidae). The impact of BTV on animals and animal products trade is enormous. In the Mediterranean basin, the principal vector of BTV is *Culicoides imicola* Kieffer, although *C. obsoletus* and *C. pulicaris* complexes are suspected vectors, too. Only in USA BTV has caused annual losses of US$ 125 million. BTV is classified as a List A disease by the Office International des Epizooties. Susceptibility and dissemination of viruses in *Culicoides* is determined by environmental factors.

Italy, because of its strategic position in the centre of Mediterranean sea, has a fundamental role in several kind of immigration, as evident in all story of Europe. Italian peninsula in the last 30 years was the crossroad of a massive clandestine immigration to Europe from other continents. Also in this exact moment, in some part of Asia (Bangladesh, Syria, Pakistan, Turkey) or Africa (North Africa, Ethiopia, Somalia, Congo) a tsunami of desperation is moving with any kind of transportation to engage a terrible and dangerous journey of thousands and thousands of kilometres to at the end perhaps break any hope at few metres from the coast or pass years impotent and trapped in the welcome centres or be persecuted for clandestine entrance. They are escaping from a hell of wars, pestilences, food deficiency, political and racial persecution. They have two main destinations to reach the Italian soil: on one side in the Tyrrhenian sea the island of Pantelleria, in the Sicily channel and distant only 62 miles from Africa, and on the other site in the Adriatic Sea the coast of Puglia, in the South of Italy.

By the advanced techniques of genoma changes, it was possible to trace the pathways of BT diffusion in Europe, mainly consisting in two different routes . Now looking at **Figure 1** obtained by sequence analysis of BTV serotypes, reporting the routes of BTV to Europe, we have exactly the some previous routes (except Sardinia). Thus migration of insect vectors and human migrants follows exactly the some passages. Off course it does not mean that migrants are responsible of the insect invasions, but that these situations are common and determined by geographic and climate conditions. Thus the key argument is understand the reasons of migration and epidemic explosion. If for human migration we know there are social, political and economic situations, we must now know the pushing effects in case of insect-born bacteria.

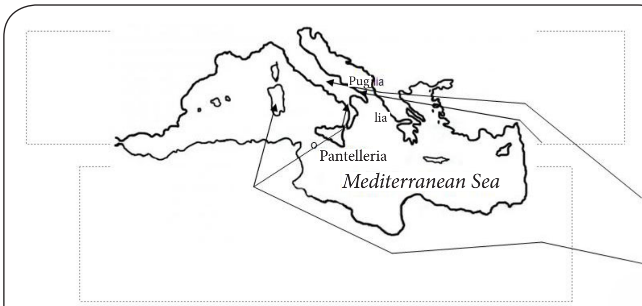

Figure 1. The two main routes of BTV vectors diffusion as reported by Purse et al., [13]: on one side in the Tyrrhenian sea the island of Pantelleria, in the Sicily channel and distant only 62 miles from Africa, and on the other site in the Adriatic Sea the coast of Puglia, in the South of Italy. The same routes are actually used by migrants to Europe by the Italian soil.

In Sardinia (the second main island in Italy) from antiquity sheep breeding have been one of the main activity for the population, especially for production of a high quality and very appreciated cheese, named *pecorino* from *pecora* that means sheep in Italian language. Therefore sheep are crucial in Sardinian economy, like in Scotland or Australia. In Sardinia the winter are particularly mild and summer hot and dry, and these conditions increased during the last decades. Thus we have the mix of a lot of sheep and positive climate conditions.

In the last decade multiple epidemics of Bluetongue occurred in Sardinia resulting in the loss of >500,000 sheep [33]. One strategy against the vector is the classic direct control of adults through the application of insecticides in infected holdings and onto the back of the animals. However, this approach resulted ineffective, while a mass vaccination campaign gave in a significant reduction in clinical cases of the disease but with evident difficulties in cost and diffusion. The other approach consisting in controlling *Culicoides* larvae has been so far not approached because the breeding sites of *C. imicola* were poorly identified and characterized, but well known by shepherds.

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The key argument is that *C. imicula* selectively breed in moist microhabitats, such irrigation channels, drainage pipes and dung heaps. Therefore, as a matter of fact in Sardinia *C. imicola* is present in muddy habitats lacking surface water, such as those found adjacent to leaking watering troughs and along pond margins contaminated with animal faeces [34]. Other approaches are necessary. The possible use of neem cake was tested and based on three considerations:

a) the control of adult insects is very difficult, since they are able to rapidly widespread in a large area, passively be dispersed by the wind; therefore the other chance is to attack at the larval stage when the insect is concentrated in particular mostly habitats.

b) the insect is already widespread interesting large areas that have to be treated with a low cost and ecofriendly product.

c) collaboration of population is essential and more effective than government campaigns of disinfestations; most of the larvae must killed and rapidly, so that the effects are evident in order to convince shepherds, farmers and breeders to collaborate in a territory that only them really know.

The assays concerning the larvicidal activity of neem cakes were conducted by the team of the University of Sassari, Italy, in collaboration with the University Sapienza of Rome, using a commercial neem cake containing low percentages of limonoids (3,750 ppm of azadirachtin A+B, 7,980 ppm of salannin and 1,850 ppm of nimbin) determined by HPLC and HPTLC analysis. Laboratory bioassays were conducted with neem cake on larval mortality of *Culicoides* in water after 7 days, affording a lethal concentration value (LC_{50}) of 0.37 g/l was obtained. Further the treatment was applied directly in field with neem cake at dose of 100 g/m^2 in a larval breeding site of *Culicoides* located in a riverside of a pond margin of a livestock farm in Sardinia. The result was a significant reduction in *Culicoides* emergence, recorded until 28 days. Therefore, *C. imicola* resulted highly sensitive to the neem cake and higher effected in comparison with *C. cataneii, C. circumscriptus* and *C. festivipennis*, that resulted effected in lower extents [35].

Emergency in central Italy: The tiger mosquito

Arbovirus (ARthropod-BOrne Virus) are another group of viruses that are transmitted by arthropod vectors. Many important diseases are related to some Arbovirus, like Dengue, Chikungunya fever and West Nile virus disease, and recently several infections, like the yellow fever, transmitted by *Aedes* sp.

In the last decades, the spreading of Asian Tiger, *Aedes albopictus* (Skuse) (Diptera: Culicidae), all around the world, has caused mainly the colonization of towns environment in Southern Europe. Until the last two decades, this insect was practically unknown in Europe, including Italy. Rapidly the aggressive tiger mosquito was able to substitute the other mosquitos, occupying every available place, but in particular man environment, dominating mainly in urban areas (flowerpot, little water pool), as reported in Figure 2, but also rural places are invaded. About the condition of the preferred habitat the situation is very similar to that already reported for *C. imicula*. However, tiger mosquito distribution was higher and therefore quite all the Italian population was directly involved.

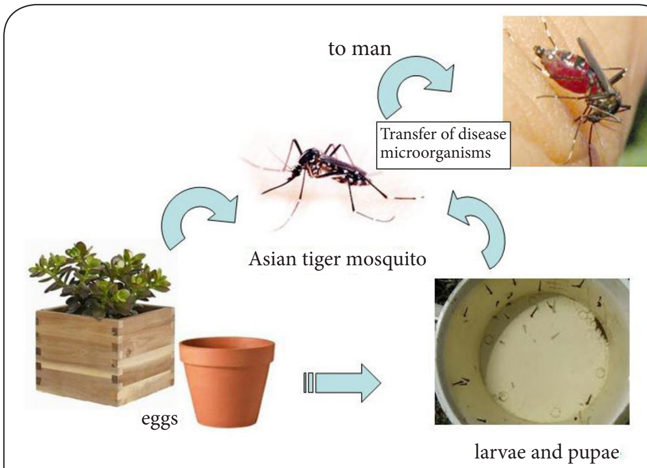

Figure 2. Diffusion of Tiger mosquito is facilitated in the urban areas by the presence of small surfaces of water (flowerpot, little water pool).

The invasion of this blood sucking mosquito raised serious concern, because its bites cause great trouble. It is a competent vector for the transmission of at least 22 arbovirus., i.e., local transmission of the *Alphavirus chikungunya* (CHIK) has been referred in two small towns in the province of Ravenna, Italy during the 2007 summer (Figure 3).

At the present, the most spread chemical insecticides used as mosquitoes larvicidae includes organophosphates, pyrethroids and insect growth regulators. In spite of the increasing of pollution by residue of synthetic pesticide in towns environment, this is an inevitable threat to citizen health, but the lack of mosquito management in private areas makes the level of infestation by *A. albopictus* out of control.

In according with the insect biology one of the most important reproduction sites chosen by the insect are saucers flower pots and road drains. A smart situation similar to that already reported for bluetongue. Owing to this situation, the usual treatment on urban areas, based on large dispersion of insecticides against adult insects, resulted ineffective, asking for new strategies. The reproduction sites could be very suitable to develop a adequate strategy using selectively botanical insecticides and asking the active capillary collaboration of the population. The strategy of the control is changing from large territories to a dispersed situation house to house.

The neem cake methanol extract was able to block the tiger mosquito at the larval stage [36,37]. Owing to the afore reported low content of azadirachtins, the total alcoholic extract was separated in extracts of increasing polarity (*n*-hexane, ethylacetate, *n*-butanol, water). The ethylacetate extract resulted

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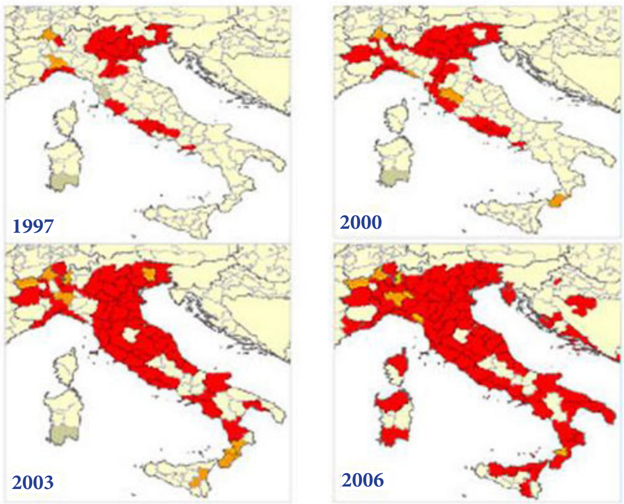

Figure 3. The diffusion of virus Chikungunya. In Italy in August 2007, the Emilia Romagna region reported the presence of a focus of epidemic infection of virus Chikungunya, near the town Ravenna. Virus chikungunya was found in Aedes albopictus, which is considered one of the most important vector.

the most active, followed by the n-hexane, whereas butanol and water resulted practically without significant toxicity [38,39]. The HPTLC analysis showed a very low presence of limonoids and therefore other compounds must be considered responsible of the reported activity [40]. Other activities of neem cake are actually under study, including antibacterial properties that can be used in meat preservation [41].

Emergency in puglia: *Xilella fastidiosa*

After the astonishing advances of the Molecular Biology, ordinary people, educated by TV investigation serials (medical ones like "Dr. House" are more realistic), believe that in few hours the lab can give any information about presence and identity of any kind of infecting microorganisms. However there are challenges very difficult and same cases remain very, very complicated to be solved.

After so many frustrating efforts to reveal the secrets of such microorganisms the scientists used to name them with the term *fastidiosa* (fastidious), to evidence their feeling. *Fastioso* (troublesome) derives from the Latin *fastidium*, which is the mix of *fastus* (arrogance) and *taedium* (meaning boredom, tedium, but also disgust).The problems raised from the difficulty to cultivate these microorganisms and to understand the environmental situation, involving the alliance with different hosts.

Most animal and plant pathogens are not able to survive, grow and reproduce outside a biotic selected environment. They are obliged parasites in host cells or organisms, often causing diseases. In particular to spread efficiently they needs a vector. A "vector" can be defined as an organism that can acquire and subsequently transmit the pathogen. Arthropods (insects and mites) are the most common vectors of plant and animal pathogens. Among them Hemiptera - aphids, leafhoppers, whiteflies, mealybugs and also thrips insects - are the most important plant virus and other pathogens vectors [42]. In Italy, many of vegetable and ornamental crops of strategic economic importance are highly damaged by diseases spread by leafhoppers, like *Impatiens necrotic* spot virus and Tomato spotted wilt virus [43].

Leafhoppers are also the vector of the so called fastidious prokaryotes [44,45]. As discussed above the denomination derives by the peculiarity to be not cultivable on growth media. This is determinant limit to study these microorganisms and develop efficient control strategy. Two XLB (xylem-limited bacteria), *Xylella fastidiosa* and *Pseudomonas syzygii*, are transmitted by sucking insects that feed on xylem sap but are not transmitted mechanically from plant to plant. These XLB occupy a highly specialized yet diverse ecological niche: xylem, the water-conducting system typical of plant hosts [46,47].

Actually there is no efficient control of fastidious prokaryotes diseases of plant and crops, while viruses can be eliminated by micro-propagation technique and new health plantlet re-obtained. Furthermore, insect vectored plant viral and bacterial diseases are of big concern for handling of seed and vegetative propagation of high quality crops (vines, olives, fruits) because the farming operations such as pruning and grafting amplify the action of the vector contributing to the transmission of the pathogens. Often the unique measure to counteract the epidemic spread of viruses and viruses like plant diseases consists into a drastic intervention with the eradication of the all involved plants [48].

The incidence of these diseases is strictly dependent by weather. Each environmental change, whether occurring as a natural phenomenon or through human intervention, changes the ecological balance and context within which disease hosts or vectors and parasites breed, develop, and transmit disease. In adapting to changed environmental conditions, including reduced nonhuman population and increased human population, some vectors display conversion from a primarily zoophyllic to anthrophyllic orientation.

In temperate regions seasonality is a key factor of mosquito-borne diseases. In most parts of Europe summer temperatures are as least as high as in warmest seasons of must of the Tropics. The crucial difference lays in the cold winter. Mosquitoes native to temperate regions had evolved strategies to survive to the adverse period, as well the connected pathogens. A similar situation occurs in Tropics, but to face the unfavorable dry periods. If mosquito-borne pathogens are by chance introduced during the hot season they are favored in survival by the temperature and helped in diffusion by the vector, but they are eliminated by cold winters. If the climate situation changes and hotter winters and dry conditions repeat for years, mosquitoes of Tropics can survive and propagate together with their pathogens. Temperature affects their rate of multiplication in the insect. It is not only a survive problems, stability can allow, beside the diffusion, the evolution of the pathogens. Thus a single place, in minimum point of the geography, a neglected part of the world, a little change of the pathogen genome can give rise to a new type of organism, a deterrent form of life never appeared in the Universe. If the natural experiment is successful that depend by the climate conditions.

In the summer 2013 a large quantity of olive tree cultivation in Puglia region (Southern Italia) was devastated by the infection of *Xilella fastidiosa* [49,50]. Puglia is one of the Italian main producer of olive oil. Olive trees are everywhere as fundamental part of the typical Mediterranean environment. Therefore the importance of olive tree is not only a economic but a habitat, historical, cultural aspect. Now the landscape changed in hundreds and hundreds of hectares. Thousands of secular trees appear totally destroyed and dehydrated. The presence of *Xilella* in the region is well known by at least 30 years but so far it was considered one of the several diseases that can affect olive. In 2010 the epidermis started in a little place near the town of Gallipoli and diffused like wildfire. The models suggest that, continuing the mild winters of the last years, the disease will propagate and other regions, like Calabria and Sardinia, should be interested in 2014.

The control appears complicated being complex the mechanism of *Xilella* diffusion. *Homolodisca vitripennis* or *Cicadella viridis* or other Cicadellidae are considered the main insect vector, although other species could be involved. Infection is also supported by some micro molds pertaining to the genus *Phaeoacremonium* and take advantage of the collaboration of larvae of lepidopterus *Zeuzera pyrina*. The larvae help the bacteria invasion trilling the xilem and generating a net of microchannels. Thus we have a complex network involving at least four different organisms, to which we should add the human efforts to control the pest. Apparently a system too complicate, but it works, efficiently. The first problem to face is where the change, generating the epidemic invasion, started. Perhaps a change of the bacterial genome could be involved generating a more aggressive mutant, or an increases diffusion of the insect vector or of the alleated ally lepidopteron. Nowadays each step is under investigation in the hope of finding out the Achille's heel of *Xilella*. Meanwhile, considering the speed of the diffusion of the epidemic disease, a program to use the effect of neem cake in the control of the insect vector at the larval stage is starting on the basis of the positive larvicidal experimental data evidenced by neem products. In any case the environmental changes are considered a key cause by everyone.

Conclusion

Environmental changes are argument of actuality, together with the limits to growth. The two arguments are considered crucial for mankind future. The main concern is the environmental changes and effects of human activity can not be efficiently followed by our genome changes. Changes are often too fast. Our genome remains almost unchanged, considering a mutation rate of approximately 0.3% per million years [51]. Our genetic background is not able to keep up this increasing mismatch. In this regard, microorganisms have a great advantage, being able to change their genome more rapidly.

However we have a very important counteraction. The history tells that we can use technology as decisive tool. Technology is our only possibility to face changes and their consequences. However, although we possess a high level of technology, the results are scattered and divided in a plethora of disciplines and specialists. Any real utilization for any new need or necessity is a challenge of interdisciplinarity researches.

The three reported examples evidence the impact of clime on key production and lifestyles, as well as the need of new control strategies, including the use of natural products. Depending by the policy in capacity and speed in counteracting, precious Mother Nature gives will be still available or lost for ever, as in the cases of olive oil or sheep wool.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	MN	KM	PD
Research concept and design	✓	--	✓
Collection and/or assembly of data	--	✓	--
Data analysis and interpretation	✓	✓	✓
Writing the article	✓	--	--
Critical revision of the article	✓	--	--
Final approval of article	✓	✓	✓
Statistical analysis	--	--	✓

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