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Future and Prospect use of Pyrethrum (Chrysanthemum cinerariifolium) as Part of the Integrated Pest and Disease Management (IPDM) Tool in Turkey

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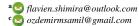
ABSTRACT

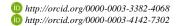
Review Article

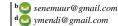
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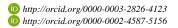
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Nowadays, immediate environmental friendly solutions such as the use of biopesticides and other methods to control and manage pests are well needed. They are imperative due to the continuous accumulation of toxic residues from synthetic insecticides into the environment, the contamination of global agro-ecosystem and resistance of certain insects and pathogens. The global renewed interest of botanical pesticides does not leave aside Turkey. Thus, many environmental reports pointed out contaminations in different regions of Turkey by chemical pesticide residues, like lambda-cyhalothrin in some conventional grapes farming in the Aegean region. The use of botanical pesticides like Pyrethrin extracted from Pyrethrum's flowers (Chrysanthemum cinerariifolium) is part of sustainable agriculture goals to reduce the incidence of pests and diseases without any alteration to the natural balance. The purpose of this review is to analyse and identify the possibilities of Pyrethrum production in Turkey. Various research works around the world were compiled and some key informants were correlated to existing researches in Turkey. Subsequently, it was found that the Black-Sea region of Turkey has great potential in the growing and industrial production of Pyrethrum by its weather patterns (temperatures, pluviometry and humidity) and soil characteristics. The region has a similar climate with the East-African high-lands well-known for Pyrethrum production. Thus, the country has shown great technological advances and the capacity to produce vegetative and disease-free clones for other ornamental plants by using tissue culture techniques. Therefore, the multiplication of high-quality vegetative clones of Chrysanthemum cinerariifolium and their maintenance can sustain commercial and long term production of Pyrethrum in Turkey.











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Introduction

It has been reported that many pests, diseases and weeds cause considerable yield losses and genetic resource damages in countless plant productions (Bayrak, 2010). Plants have developed different mechanisms to protect themselves against pests throughout evolution. These mechanisms manifested in plants by some biochemical events and morphological changes. Thus, plants synthesize secondary metabolites which can affect pest behaviour and physiology in different levels of inhibition and toxicity. They initially provide high plant resistance to pathogens and herbivores (Dubey et al., 2010). The most important secondary metabolites synthesized by plants are; alkaloids, glycosides, phenols, terpenoids, tannins, and saponins (Shanker and Solanki, 2000). These synthesized metabolites have been used in agriculture for many years within the scope of pest control and management. Before the discovery and use of synthetic insecticides, it was wellknown that plant-based insecticides play an important role in agriculture. However, synthetic insecticides have been more effective than plant natural insecticides and their effectiveness prevails for a long period which has led to their use for many years. In the past few years, it has been scientifically demonstrated that the unconscious use of synthetic insecticides harms nature, environment and human health. Besides, it was found that pests develop resistance against these insecticides. For these reasons, a renewed interest in plant-based insecticides has reemerged and more studies have been undertaken in this current decade (Güncan and Durmuşoğlu, 2004). Although many plants are known to have an insecticidal effect, very few are used in insecticides production (Isman, 1997). The reason for this is due to the lack of sufficient natural

resources, lack of standardization and difficulty to obtain a production license as reported by Güncan and Durmuşoğlu (2004).

One of the plants with insecticidal production ability is Pyrethrum (Chrysanthemum cinerariifolium) which is a part of the chrysanthemum genus in the Asteraceae family. It is known to have originated from the Balkan and Eastern coasts of the Adriatic Sea in the Dalmatia region of current Croatia. Its natural habitat can be extended to Bosnia and Herzegovina, Montenegro and to Italy (Zito, 1994; Hitmi et al., 2000a; Grdiša et al., 2009; Tóth et al., 2012; Jongschaap et al., 2018). This perennial ornamental plant is also named Tanacetum cinerariifolium (Trevir.) by some biologists (Li et al., 2014). It is a diploid plant with 18 chromosomes (2n=2x=18) as reported by Liu and Gao (2007). There are more than 40 species within chrysanthemum genus and to large extent, they are found in East-Asia, particularly in China. Chrysanthemum cinerariifolium is the most popular one. Apart from being used as an efficient insecticide, it is also used in medicine. The plant is characterized by white-yellow flowers that grow to a height of about 60 cm. One particular feature of its flowers is the existence of four different active components like, Cinerin-I, II Pyrethrin-I, II and Jasmolin-I, II which are major insecticidal constituents of pyrethrins. They are especially found in oil glands onto the seed inside the thoroughly packed flower head. In other parts of the plant, Pyrethrin can be also found in small concentrations (Zito et al., 1983; Grdiša et al., 2009; Zhao et al., 2009; Liu and Gao, 2010). As described by Hitmi et al. (2000a), pyrethrins are a set of monoterpene esters with a close similar structure and they result from esterification of two monoterpic acids (pyrethric acid and chrysanthemic acid) with three ketone alcohols (cinerolone, jasmolone and pyrethrolone). In the general effort toward sustainable agriculture, natural insecticides are used as ecological and environment-friendly in the management of agricultural pests. Almost 80% of natural insecticides produced globally are extracted from Pyrethrum. Thus, Pyrethrum is seen as a legitimate alternative to synthetic insecticides and its extracts contain active compounds that are effective to control different pests including insects and plant diseases' vectors. Apart from Pyrethrum, other well-known botanical pesticides with less damage to the environment are: Neem (Azadirachta indica) pesticides which are systemic and do not leave residue on the crop, Azadirachtin which is non-toxic to mammals, pollinators and fishes, Sabadilla or Cevadilla extracted from Sabadilla lily's seeds (Schoenocaulon officiale), Carvone or Tallent from Caraway's (Carum carvi) essential oil which is often used to protect potatoes in storage against bacterial rotting (Dubey et al., 2011).

In this review, the possibility of Pyrethrum introduction and its adaptability in Turkey are being examined. The rising need for natural insecticides in the country and a cool temperate climate suggest and imply future opportunities in the mass cultivation and growing of Pyrethrum and other plants from which natural insecticides are extracted. Pyrethrum growing has been tested for the first time in Turkey in 1928. It was brought in as seeds from foreign countries and it continued to be cultivated till losing its value among producers despite obtaining high-quality yield. Pyrethrum cultivation was tried again for the second

time by the Ministry of Agriculture initiative at Bornova Plant Protection Station and positive results were obtained. By incentive actions by the Turkish government, free saplings were distributed to the producers, unfortunately soon later its production was abandoned for an unknown reason (İpekçioğlu and Aksu, 1943). The active compounds in the Pyrethrum are called "pyrethrins" (Tanker and Tanker, 1990). Since the introduction in many countries of the new legal limitations and requirement of chemical residue tolerance in food, natural substances that are less toxic and do not cause problems gained importance. And doubtlessly a recognition was likewise drawn to the pyrethrins. In Turkey, the Ministry of Agriculture has proposed the production and use of products containing pyrethrin. It was also thought that both the chemical residue problem would be solved and the country would earn economically, but this project could not be implemented for financial reasons (Arslan and Yılmaz, 1993).

Ecological growing conditions of Pyrethrum

Climatic Conditions

In East-Africa and particularly in major producing countries; Kenya and Rwanda, Pyrethrum is always found and grown in highlands with an altitude between 1,500 and 3,000 meters above sea level. In Kenya, highlands are located in the Western regions of the country. In Rwanda, they are located in the Northern regions. Those highlands are suitable for Pyrethrum cultivation and they are characterized by annual rainfall between 1,000 and 1,400 mm. The optimum temperature observed in Kenya lies between 15 and 20°C. Those temperatures are ideal for maximum photosynthesis. The same ranges are being noticed in Rwanda with an average annual temperature of 15.7°C (with a minimum of 11.6°C and a maximum of 20°C) (Wandahwa et al., 1996; Kagabo et al., 2013). In Australia, similarities in temperatures with East-Africa are being observed in the main growing state, Tasmania (in the North-West of the state). Tasmania has a warm, temperate maritime climate (four distinct seasons). Temperatures are moderate with 20°C maximum during summers and 13°C minimum during winters. Rainfall figures are low compared to East-Africa with 750 mm of annual rainfall (Greenhill, 2007). It was reported that the increase in flower production is related to temperature variations between night time and daytime which are low (under 13°C) and warm (15-20°C) respectively. Likewise, a rotation between sunny periods and rainfall periods are the suitable conditions for pyrethrum cultivation. It was found that the pyrethrins' content of the flower is affected considerably by temperature. Thus, temperatures above 21°C inhibit flowering completely along with frost (extremely low temperatures) that damages Pyrethrum (Kushwaha et al., 2012; Wandahwa et al., 1996).

Soil Conditions

Pyrethrum is generally grown on fertile and well-drained soils such us loamy soils (derived from volcanic rocks) of Kenya and Rwanda and also krasnozem soils (derived from basalt parent rock) seen in Tasmania (Greenhill, 2007; Mureramanzi and Bizimungu, 2015; Wandahwa et al., 1996). Loamy soils are known for their

good structure that results in exceptional water infiltration and holdings. The Pyrethrum rooting system can reach a maximum depth of 50 cm. It is generally found within the top 30 cm of depth. Pyrethrum can grow on soils with great pH ranges from gravelly to slightly alkaline and also on slightly saline (or calcareous). In Kenya, it is found in soils with pH values between 4.0 and 6.3 while in India, it is grown in soils of 3.0 to 5.1 of pH values. It was revealed that Pyrethrum can display soil acidity tolerance between 5.3 and 6.0 of pH. The suitable and recommended soil pH should be slightly above 5.6 for nutrients availability and accessibility to the crop (Wandahwa et al., 1996). One of Chrysanthenumm cinerariifolium close relatives; Painted Daisy (Pyrethrum roseum or Chrysenthemum roseum) is found in the natural habitat of Erzurum province in the Eastern Anatolia region of Turkey (Tanker and Apaydın, 1973). By assessing soil features in the Erzurum plain of 22 different surface soil samples, Yıldız and Bilgin (2008) revealed that the pH of the soil was between 6.86 and 8.82. They also determined that the organic matter content ranged between 0.46 and 4.49% and the lime content ranged between 1.01 and 13.97%. In addition to being found in the mountainous area of Erzunun, Pyrethrum roseum is also naturally blooming in Corum and Ardanuç, two zones of the Turkish Black Sea region (Davis, 1975). It was determined that in the aforementioned region, known for tea production, the average soil pH is 4.14, 3.72 and 4.31 in Artvin, Rize and Trabzon provinces respectively (Özyazıcı et al., 2013). Other similar studies were conducted in the same region. For instance, in the study carried out in Trabzon district of Trabzon province, Gür (2019) has revealed that the soil pH was 5.29, the lime content (CaCO3) as 2.12%, total salt rate as 0.016% and organic matter rate as 2.78%. Likewise, in the Kalkanlı mountainous region of Trabzon, a study was conducted by Gedik (2017) and he found that the pH value of the soil was 5.46, the organic matter content was 2.20% and the texture of the soil was silty-loam soils with 0.010% of salt concentration. In two different studies conducted in the Hayrat district of Trabzon province, it was determined that the pH of the soil was between 4.65 and 4.96 and the associated amount of organic matter was 4.21% and 5.34%, respectively. The soil texture was found to be clayloamy (Aydın, 2008; Kuru, 2009). Those types of soil in the Black Sea region can be excellent for Pyrethrum growing due to soil acidity similar to those in countries like Kenya, India, and Rwanda. In an effort to increase considerably Pyrethrum flower yields, Rajeswara Rao et al. (1983) advanced that particularly in acid soils where the availability of phosphorus limits the Pyrethrum yields the application of phosphatic fertilizers up to 120 kg P₂O₆ ha⁻¹ year-1 is necessary. Besides, nitrogen and potassium fertilizers don't change Pyrethrum flower yields and pyrethrins concentration.

Global Production of Pyrethrum

Pyrethrum is used as ornamental and landscape plants for its flamboyant flowers. The flowers of the Pyrethrum plants are appreciated for their pyrethrin extracts. Also, Pyrethrum provides an insecticide widely used worldwide and in Turkey. Natural insecticides are so popular because "insect resistance" has emerged from the use of synthetic insecticides against pests. Environmental damages are also correlated with the use of synthetic insecticides (Göktürk, 2017). Pyrethrum has been produced since time immemorial. Pyrethrum production in the world has been dominated by the following countries: Tanzania, Rwanda, Papua New Guinea, Ecuador, Kenya, and Tunisia. Besides these countries, Italy and Morocco are also important producer countries. While Japan is among the top producers, production has been decreasing in recent years. If we look at the 2020 data, the uncontested world production leader of dried Pyrethrum flowers is Tanzania as shown by the last three years recorded data (Figure 1). In 2018, Tanzanian Pyrethrum was grown to a total production area equal to 13,941 hectares and 7,036 tonnes of dried Pyrethrum flowers were produced (FAO, 2020).

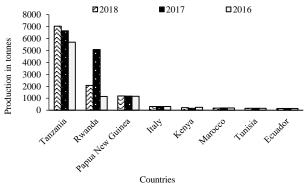


Figure 1. World major producers of dried Pyrethrum flowers in year 2016-2017-2018 (FAO, 2020)

Natural Pyrethrins: Active Compounds in Pyrethrum

Pyrethrins (Pyrethrin I: C₂₁H₂₈O₃, pyrethrin II: C₂₂H₂₈O₅) are natural insecticides with a well-known ability to repel insects (Dent, 2000; Shivanandappa and Rajashekar, 2014). Significant concentrations of pyrethrins are usually spotted in flowers. Thus, the dried flower's powders contain natural pyrethrins too (Matsuda et al., 2005). Their repellence is effective in agriculture and horticultural crops to contain numerous vectors of disease such as insects. They have been used successfully against the Mexican bean beetle (Epilachna varivestis) on soybeans. Natural pyrethrins are extracted from the flowers of Pyrethrum and their only burden is that they are less stable biochemically than their synthetic counterparts, pyrethroids. Famous pyrethroids are Deltamethrin and Allethrin (Dent, 2000; Hitmi et al., 2000a). Thus, synthetic pyrethroids have also a disadvantage to be less persistent and promptly biodegradable. A high contact activity is discerned in pyrethroids and they are specifically effective against lepidopterous larvae. Some of the synthetic pyrethroids have moderate toxicity to mammals and they can be particularly harmful to fish and non-target invertebrates (Dent, 2000). Pyrethroids are not specific in their actions and since they stick to leaf surfaces for long periods, disease-causing insects and useful ones are killed. They are also poisonous to honey bees. This makes them less environmentally friendly than pyrethrins (Dubey et al., 2011). Pyrethrins have an instantaneous effect on insects at low concentrations and they can wipe out most insect species (Bailey et al., 2010). Insects are affected by pyrethrins through direct contact, inducing a disruption in their nervous system. Transmission of an insect's nerve impulses is terminated through the disruption of sodium and potassium ion-exchange process (Dubey et al., 2011; Shivanandappa and Rajashekar, 2014). Its application in low doses may result in convulsions and brief paralysis of the insect. Subsequently, synergistic interaction with other compounds is required for effectiveness and persistence. So often, synergist such as piperonyl butoxide (PBO) or noctyl bicycloheptane dicarboximide is used (Dubey et al., 2011).

Environmental Impact of Chemical Pesticides in Some Regions of Turkey

It has been reported that in the regions with a long tradition use of pesticides, it is more likely to found pesticide residues in soils, food and freshwater supply. Thus, this situation leads to a certain and adverse impact on the environment and human health. In the study undertaken by Turgut et al. (2011) in the Aegean region (Izmir, Manisa and Denizli provinces) where the majority of table grapes are produced, it was found that in conventional vineyards pesticide residues were present. The most commonly found, in different proportions and all three provinces were; chlorpyrifos-ethyl, chlorpyrifos-methyl, deltamethrin and lambda-cyhalothrin followed in fewer quantities by Chlorpyrifos-methyl and deltamethrin. Likely, no pesticides were detected in vines from organic vineyards where farmers have adopted integrated pest management. In the risk assessment of those pesticides, only lambda-cyhalothrin exceeded maximum residue level (MRL) of pesticide exposure established by Pesticide Safety Directorate (PSD, York, UK). Other pesticide residues did not exhibit any risk. Other pesticides with negative impacts on the environment are; Organochlorine pesticides (OCPs) and Organochlorine and polychlorinated biphenlys (PCBs). They were globally used since the 1940s as the first synthetic organic pesticides in the control of pests and diseases. Afterward, due to their proven carcinogenetic/mutagenic potential and their toxic effects on animal development, immunological, and reproduction, they were internationally banned from the markets. In Turkey, the same restrictions have been implemented since 1983. Nonetheless, their relatively high concentration presence in some Turkish environments suggest a continuous usage by farmers (Ayas et al., 2007; Chen et al., 2007; Kalyoncu et al., 2009).

Kalyoncu et al. (2009) carried out a study to investigate the levels of OCPs residues in fish species sold in the central Anatolia province of Konya. Thus, fishes sold in the region are from the seas surrounding Turkey (Aegean Sea, Mediterranean Sea, and the Black Sea) and also from Beyşehir Lake located in 90 kilometers of Konya city. In total fourteen different organochlorine pesticides (δ-HCH, α-HCH, β-HCH, γ-HCH, Heptachlor, Aldrin, Heptachlor epoxide, α-Endosulfan, p-p'-DDE, Dieldrin, Endrin, β-Endosulfan, p-p'-DDD, p-p'-DDT) were analyzed in eighteen different fish species (bluefish, bonito, common seabream, garfish, gilt head bream, goby, gray mullet, greater amberjack, gurnard, horse mackerel, import mackerel, native mackerel, pilchard, pike perch, red mullet, sea bass, trout and whiting fish) by using gas chromatography with electron capture detector (ECD). The most predominant pesticide contaminants in fish muscles were DDT and HCH. Nearly all samples, detectable levels of HCH, aldrin and heptachlor were discovered. Luckily there was no suggestion of important health risks linked with the consumption of these fishes. All the above-listed risks and contaminations by synthetic pesticides in different regions of Turkey illustrate real needs in the adoption of new agriculture practices which require the use of biopesticides less harmful to the environment.

Pyrethrum in Organic Farming and Pest Management

The negative effects of chemicals and their impact on human and public health are increasing progressively. Organic farming and good farming practices are being carried out to eliminate the effects of all these negativities on human health and nature. Organic farming constrains the use of synthetic pesticides, hormones, and chemical fertilizers. Thus, it is mainly aiming to restore the natural balance of nature devastated by improper use of chemicals in nature. Furthermore, the use of organic fertilizers in organic farming is recommended to boost the resistance of plants against plant natural enemies and for the preservation of the soil (Hekimoğlu and Altındeğer, 2006). It has been discovered that approximately 1,500 plant species and varieties have an insecticidal effect (Erdoğan, 2013). Nowadays, over 2,000 herbal extracts are obtained from different sections of plants such as roots and seeds. Thus, their effectiveness against pests has been recognized (Öncüer, 2000). Plant-based insecticides are extracts of plants and they are used in agricultural applications, such as; Pyrethrum, Nicotine, Quassine, Capsaicin, Sabadilla, Ryania, Azadirachtin, Rotenone, Garlic, Melia azedarach L., etc. (Table 1). The most used plants for their insecticidal extract are neem (Azadirachta indica) and Chinaberry (Melia azedarach L.) (Erdoğan, 2013). The low residual effect is one of the advantages of using pyrethrins in organic farming. During postharvest applications, for instance, on grains, it has been revealed that in a period of three to four months of storage, 50% or more of the applied pyrethrins disappear (Gallo et al., 2017). Pyrethrin is environmentally safe and highly biodegradable due to its instability on light, high temperature, oxygen and water. It is recognized and approved in the European Union regulation (EC) No. 834/2007 on organic production and labeling of organic products. Consequently, it is one of the leading and used insecticides in organic farming systems (Grdiša et al., 2009).

Management of pathogens and diseases in Pyrethrum

Like other ornamental plants, Pyrethrum is not exempt from pests and diseases. Diseases affecting Pyrethrum do prevail in both Pyrethrum production systems; in the low-input system like in East-Africa which is characterized by limited mechanization and where at certain extent no production inputs (such as herbicides, fungicides, and fertilizers) are used and also in high-input or highly mechanized Pyrethrum production systems like Tasmania-Australia. Pethybridge et al. (2008) have described major diseases and their related prevalent pathogens (Table 2) affecting mainly production in Tasmania.

Table 1. Important botanical pesticides and their targeted pests

Plants	AC	Targeted pests	Mode of action
Neem (Azadirachta indica)		Insects (Homoptera, Heteroptera, Lepidoptera, Coleoptera, Diptera, Hymenoptera and Orthoptera)	It acts as repellent, antifeedant, chemosterilant, and insecticide. It also has fertility reducing action by preventing egg-laying and disrupting growth and development.
Pyrethrum (Chrysanthemum cinerariifolium) Rotenone (Lonchocarpus sp.), Derris (Derris sp.) and Tephrosia (Tephrosia sp.)		Insects	Effective by contact.
		Insects	It acts as both by contact and stomach poisoning of insects.
Nicotiana tabacum; Nicotiana rustica; Nicotiana sylvestris; Anabasis aphylla	N	Insects	It enters through the tracheal system in the gaseous forms, causing paralysis and then death. It is an effective fumigant.
Ryania speciosa	RY	Insects such as; <i>Cydia pomonella</i> Lin., Archips (Lep:Tortricidae), European corn borer (<i>Ostrinia nubilalis</i>) pyralid moths (Lep: Pyralidae).	It affects the muscular system of insects and causes paralysis and then rapid death.
Sabadilla (Schoenocaulon officinale)		Heteroptera	They disrupt the functioning of sodium channels found in the membrane of the heart, nerve and striated muscle cells in the insect. It causes paralysis and then death of the insect.
Amargo (<i>Quassia armara</i> L.) and Jamaican Quassia (<i>Picrasma excelsa</i>)		Aphids (Hemiptera: Aphididae); <i>Hoplocampa</i> spp., (Hym: Tenthredinidae); <i>Bemisia tabaci</i> (Hom: Aleyroridae); Anthonomus pomorum (Col: Curculionidae)	It acts by contact and by stomach poisoning of insects.
Garlic (Allium sativum L.)		Insects such as; cowpea weevil (Callosobruchus maculatus (Col: Bruchidae)), Ephestia kuehniella and Plodia interpunctella (Lep: Pyralidae); Sitophilus oryzae, S. granarius (Col: Curculionidae), Plutella xylostella (Lep: Plutellidae), Trichopis nucleus (Lep: Plutellidae), Rapae (Lep: Pieridae).	Generally, it is a repellent and effective against pests of stored products.

AC: Active compound, A: Azadirachtin, P: Pyrethrins, R: Rotenone, N: Nicotine, RY: Ryania, S: Sabadilla, Q: Quassin, Neoquassin and Picrasmin, Source: (Kılıç 2019; Isman 2006)

Table 2. The main diseases damaging Pyrethrum production

C/R		Pests	Cymptoma
C/K	Diseases	Pests	Symptoms
A/T	Ray blight	Phoma ligulicola Baker, Dimock and Davis v. Arx. var. inoxydablis	Necrosis of the ray florets of the flowers resulting in the death of flowers and buds. They can also cause necrotic lesions that generally extend from 20 to 30 mm on the peduncle of the unopened bud.
	Sclerotinia crown rot	Sclerotinia minor Jagger and S. sclerotiorum (Lib.) de Bary	Crown rot through myceliogenic germination.
	Sclerotinia flower blight	S. sclerotiorum	Crown rot through myceliogenic germination.
	Botrytis flower blight	Botrytis cinerea Pers.	Crown rot through myceliogenic germination. Wilting and death of the entire plant
	Root rot	Lesion nematodes, <i>Pratylenchus</i> spp.	
	Tan spot	Microsphaeropsis tanaceti sp. nov.	There are tan-coloured spots that coalesce around the margins of leaves.
	Winter blight	Alternaria tenuissima (Nees:Fries) Wiltshire	Necrotic lesions which are colonized by secondary fungi. Small necrotic spots (generally <0.5 cm in diameter) on leaves.
	Pink spot	Caused by <i>Stemphylium botryosum</i> Wallroth	Lesions on leaves that exhibit necrotic halos surrounded by pink/brown margins.
	Diseases Caused by Nematodes	Lesion nematodes (<i>Pratylenchus</i> spp.) particularly <i>P. crenatus</i> , <i>P. penetrans</i> and intermittently <i>P. neglectus</i> and <i>P. thornei</i>	Significant injury to Pyrethrum
	Tomato spotted wilt virus (TSWV) infection	Tomato spotted wilt virus (TSWV)	Symptomless
	Wilt diseases	A range of <i>Fusarium</i> spp., but primarily <i>F. oxysporum</i> Schlechtend.	
	True bud disease	Ramularia bellunensis Speg	
	False bud disease	R. bellunensis	Death of buds
=	Diseases Caused by Nematodes	Root-knot nematode (Meloidogyne hapla) and lesion nematode (Pratylenchus penetrans)	Symptoms of root-knot nematode injury include large galls on roots, hairy and stubby roots. There is also severely restricted root growth, which can lead to plant death in susceptible cultivars.
	Ray Blight	<i>Phoma ligulicola</i> var. <i>ligulicola</i> and <i>P. ligulicola</i> var. <i>inoxydablis</i> .	Minor damage on Pyrethrum

C/R: Country/ Region, A/T: Australia/Tasmania, K: Kenya, Source: Pethybridge et al. (2008)

Table 3. Recent developments for *in vitro* culture of Pyrethrum

Aim of the study	Explants	Basal Medium	Growth regulators	Remarks	Reference
The effects of growth regulators during <i>in</i> vitro culture	Petiole	MS	2,4-D, BA & NAA	2,4-D promotes callus growth. BA and NAA do not affect growth.	Obukosia et al. (2005)
Buds and roots induction	Epicotyl and hypocotyl	MS	BA & NAA	Variations of NAA concentration have a significant effect on shoot growth.	Liu and Gao (2007)
		½ MS	IAA & rooting powder (ABT)	0.3 mg L ⁻¹ of BA is efficient for root induction in the MS medium as well as 0.3 mg L ⁻¹ of NAA.	
	Leaf and petiole segments	MS	BA & 2,4-D	The optimum shoot regeneration was observed with 2 mg L ⁻¹ of NAA in the	
Direct organogenesis		B5	NAA	B5 medium. 4 mg L ⁻¹ of BA and 2 mg L ⁻¹ of NAA	Hedayat et al. (2009)
		SH	NAA	induce a maximum shoot regeneration in the MS medium.	
Effectiveness of different	Nodal explants	MS	2iP, BAP, KIN & TDZ	40 μM of BAP are more effective for micro-shoot proliferation. A moderate concentration of IBA (10	Lindiro et al.
cytokinins and auxins		½ MS	IBA, NAA & 2,4-D	μM) gives a high number of roots per explant and considerable root length per explant.	(2013)

They have also listed subsisting diseases in Kenya. Moreover, among recommendations and tips for all those diseases management, there are;

- The adoption of resistant cultivars to prevent ray blight and wilt diseases. Thus, resistant plants grant a physical and physiological barrier against spore dissemination of some fungal pathogens;
- Vegetative propagation is always advised to counter diseases like Ray Blight caused by *Phoma* ligulicola;
- Some farming practices such as overhead irrigation and the use of fertilizers mainly in the management of Sclerotinia diseases and Botrytis Flower Blight are not advised;
- Plant density should be decreased by wide spacing planting. Thus, it reduces considerably humidity and leaf wetness period which result in a decline in the rate of disease development;
- The use of nematode-tolerant with a combination of crop rotation from Pyrethrum to cereal crop (like maize) for 1 year period, are efficient in the management of *Meloidogyne hapla*.

For biological control of nematodes, it was found that two specific arbuscular mycorrhiza fungi (AMF) can be successfully used against *Meloidogyne hapla*, one of the nematodes devastating Pyrethrum plantations in Kenya. The two isolates of IMF were identified as *Glomus etunicatum* (Isolate KS18) and *Glomus* sp. (Isolate KS14). Both isolates have suppressed nematodes population by more than 50% and egg production up to 75%. They have also decreased disease severity up to 71 % and 57% by Isolate KS14 (Glomus sp.) and *Glomus etunicatum* (Isolate KS18) respectively (Waceke et al., 2001).

Micropropagation of Chrysanthemum cinerariifolium

Micropropagation programs are very useful for commercial planting of Pyrethrum. It is well known that for pyrethrum, the first flowering takes place within 12 to 15 months after seedling. Thus, it is preferable to use vegetative clones for Pyrethrum propagation which gives first flowering within 3 months (Pethybridge et al., 2008). The multiplication of high producing clones of Chrysanthemum cinerariifolium and the maintenance of its clonal purity and with its protection against diseases and environmental stresses are seen and part of classical breeding programs. In vitro clonal multiplication promotes asexual multiplication and its increase propagations of pathogen-free plants. Many researchers have focused their studies on this topic. *In vitro* multiplication of Pyrethrum has started in the early 1970s. It was an alternative to vegetative propagation by shoot cuttings Chrysanthemum cinerariifolium which had a low multiplication rate and was vulnerable to root-knot nematodes (Meloidogyne halpa) attacks. Thus, in vitro clonal multiplication promotes asexual multiplication of clones with desirable features and also delivers high propagation rates. As explants, shoot tips and/or axillary buds are used. Besides, formal tissue culture procedures are followed; explants sterilization, the use of a growth medium in which different concentrations of auxin and cytokinin were supplemented. Subsequently in the fourth and/or sixth week, a subculture is performed on the propagation medium (Hitmi et al., 2000a). An in vitro and polyploidy breeding study of C. Cinerariifolium was carried out by Liu and Gao (2007) and they successfully obtained autotetraploid plantlets with 36 chromosomes (2n=2x=36) by using filter-sterilized colchicine. The obtained polyploid lines were then kept as germplasm resources for further research. Thus, polyploid plants are very important in the development of superior varieties due to related biomass increase.

The optimum in vitro callus culture and growth conditions of Pyrethrum have been set up by Hitmi et al. (1998) and they are as follows; a half concentration of standard growth media (MS) calibrated at 5.7 pH with daylight's irradiance intensity equals to 60 μmol.m⁻².S⁻¹ (16 h photoperiod) and culture temperature set at 23°C. The synthesis and aggregation of monoterpenes in the plants are known to be linked to the irradiance intensity, mostly the white light than monochromatic blue (450 nm) or red (670 nm) light. In other words, light is a substantial regulatory factor in the growth of calli and its pyrethrin content accumulation. Other recent studies have shown new developments and optimum conditions for the in vitro growing of Chrysanthemum cinerariifolium (Table 3). Hitmi et al. (2000b) have also described a straightforward and efficient cryopreservation method of Chrysanthemum cinerariifolium shoot tips by using cryoprotectants (such as ABA, DMSO and sucrose), freezing (-196℃ liquid nitrogen) and thawing (rapid rewarming at 40°C) treatments to increase survivability of in vitro shoot tips. They have obtained satisfying results with 75% of the shoot tips withstanding cryopreservation. And after which they were fully recovered and propagated on a solid nutrient medium. There was also no loss of biosynthetic observed after cryopreservation Chrysanthemum cinerariifolium plants. Pyrethrins levels remained stable. Hence, a cryopreservation protocol of in vitro plantlet's shoot tips of Pyrethrum was established. Since a long time, Turkish researchers have demonstrated their competence for conducting successfully in vitro and breeding programs for ornamental plants such as; Begonia (Begonia spp.), Cyclamen (Cyclamen ssp.), Rose, Orchids, Carnation, Gypsophila, Lilium, Tulip, Sternbergia, Peonies spp.), Leucojum, Tulipa, Hyacinthella, Hyacinthus and Muscari. (Karaoğlu 2004; Mirici et al., 2005; Doğan- Kalyoncu, 2007; Mendi et al., 2009; Kaya, 2010; Nasırcılar et al., 2010; Uranbey et al., 2010; Alp et al., 2013; Gürsan, 2014; Yucesan et al., 2014; Uzun et al., 2014; Curuk et al., 2015; Ozel et al., 2015; Baskaran et al., 2016; Kizil et al., 2016; Hurkan, 2017; Bulut et al., 2019; Kaya et al., 2019). Thus, it confirms the national capacity to produce high quality and disease-free vegetative clones of complex plants such as Chrysanthemum cinerariifolium.

Conclusion

The promotion and use of Pyrethrum are part of the same goal to attain a durable agriculture system that diminishes chemical residue impacts on the environment. Thus, Integrated Pests and Disease Management (IPDM) and a range of other control strategies for plant protection including biological and physical controls, host plant resistance and decision support tools enter in the broad spectrum of self-regulating ecological systems performed to maintain and reduce pest damages. (Bailey et al., 2010). Pyrethrin insecticidal products have been used in IPDM strategies for years and during the last few years there has been a renewed interest due to high resistance of pests to synthetic pesticides and more restricting environmental and protection laws. Thus, pyrethrins based insecticidal

products do have a fast knockdown effect on insects and exhibit also low toxicity to mammals and other warmblooded animals (Rajeswara Rao and Singh, 1982; Grdiša et al., 2009). In the present review, we have demonstrated all technological advances and possibilities of Pyrethrum adaptability in Turkey and we have found that there is a high growing potentiality in the Black-Sea coastal region. This region is high land with high elevations and it is known for its cool climate with average temperature and high humidity. Apart from climate features, another important consideration that we examine is the possibility of mass production of high quality and disease-free vegetative clones of Pyrethrum. For future research, we highly recommend carrying out an adaptability study of several different Kenyan Pyrethrum clones and improved varieties that have been released since the 1960s from different breeding programs by the Kenyan National Pyrethrum and Horticulture Research Station (NPHRS) at Molo. Evidently, they have shown high yielding results (such as Pyrethrins concentration between 1.6 and 2.1 %) in the highlands of Kenya (with an altitude between 1700 and 2200 m). Those are; twelve clones; 4331, Sb/66/107, Ma/70/1013, Ks/70/64, Ma/71/423, Ks/75/313, Ks/72/43, L/72/26, Kr/74/443, Kr/74/223, Kr/74/122, Mo/70/1124 and three varieties which are; P4, K218 and K235 (Wambugu and Muthamia, 2009). Formerly, from the clones and/or varieties with high yielding potential of Pyrethrin will be selected for further research.

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