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BIOLOGICAL ACTIVITY OF NATURAL AROMATIC PRODUCTS FROM THREE *NICOTIANA* SPECIES AGAINST BACTERIAL PHYTOPATHOGENS

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Abstract

The objective was to assess the biological activity of the natural aromatic products concrete and resinoid obtained from different Nicotiana species (N. tabacum L., N. alata Link & Otto and N. rustica L.), against some bacterial (Xanthomonas spp., Pseudomonas spp., Bacillus spp.) phytopathogens. Test pathogens were isolated from infected plant materials and soil. The aromatic products were obtained by twofold extraction with following solvents (concrete with petroleum ether and resinoid with 95% ethanol) at high temperature and subsequent concentration on a rotary vacuum evaporator. Their biological activity was tested under in vitro conditions by the diffusion method/sterile zone diameter (mm)/. Products-specific inhibition has been found at the different species of phytopathogens. The antimicrobial activity of aromatic products was general better expressed under the influence of the resinoids. The results reveal opportunities for the development of biological plant protection products based on natural extracts of the genus Nicotiana with antibacterial action.

Key words: *Nicotiana species, aromatic products, antibacterial activity, phytopathogen.*

INTRODUCTION

The potential and benefits of using plant-derived chemicals as bioactive and therapeutic agents have been the focus of extensive research in the last decades. Those potent phytochemicals almost always exclusively embody secondary plant metabolites resulting from alkaloid, terpenoid and flavonoid biosynthesis and transformations (Aravindaram & Yang, 2011). In this view, many of the species of genus *Nicotiana* (*Solanaceae*) have revealed extensive potential as basis of various isolated bioactive substances or complex extracts (Budzianowski, 2014). In fact, *Nicotiana* species are one of the richest sources of biologically active metabolites and different aspects of their therapeutic or protective potential in human medicine have been reported (Jassbi et al., 2017; Mostafa et al., 2018; Anumudu et al., 2019). Some tobacco varieties accumulated polyphenols in amounts higher than those in traditionally proven medicinal plants with antimicrobial activity. Extracts derived from cultured tobacco (*N. tabacum* L.) have been shown to show insecticidal and antimicrobial activities (Bakht et al., 2013; Kekuda et al., 2017). Although historically most research has focused on economically important types of tobacco for cigarettes - mainly on *N. tabacum,* not as much on *N. rustica* L. and relatively less on the over 70 non-commercialized *Nicotiana* species, there are sufficient evidences that many of them accumulate specific metabolites involved in plant defense mechanisms (Jassbi et al., 2017; Bally et al., 2018). The species *N. alata* Link & Otto was found to synthesize protease inhibitors against a wide spectrum of pathogens, as well as plant defensins, specialized proteins with antifungal and antibacterial effect (Bleackley et al., 2016). These inhibitors are thought to be involved in the genetic mechanism of plant protection and

the isolated plant defensins were used to generate pathogen resistant transgenic plants (Ghag et al., 2016). The specificity of the phenological development of *Nicotiana* plants makes them extremely suitable for various bioengineering procedures, as well. Some of them have been used as model plants in the experimental production of vaccines and other areas (Budzianowski, 2014; Bally et al., 2018). The species *N. tabacum* L.*, N. benthamiana* Domin., *N. glutinosa* L. and *N. alata* Link & Otto, along with other plant species, have performed successfully in tests for the production of valuable immunoglobulins and other recombinant proteins (Budzianowski, 2014). Different extracts of these and several other *Nicotiana* species have revealed expressed antibacterial and antifungal activities against human and plant pathogens, as well as antioxidant and insecticidal properties (Ru et al., 2012; Nwachukwu, 2017; Al-Lahham et al., 2020). On the other hand, *N. tabacum* and some of the rest *Nicotiana* species can be regarded as genuine aromatic plants, and fresh or dried leaf and flowers are processed to obtain traditional aromatic products, used in perfumery, cosmetics and phytopharmacy. Those natural aromatic products include: tobacco essential oil (obtained by hydro-distillation from an acidified medium); extraction concentrates tobacco concrete (by extraction of dry material with non-polar organic solvents, such as petroleum ether, n-hexane, and concentration by complete removal of the solvent); tobacco resinoid (by extraction with polar solvents, mostly ethanol and concentration); tobacco absolute (obtained by re-extraction of concretes, resinoids or other extracts with ethanol at cooling and separation of precipitates), and some others (Bauer et al., 2001). Aromatic products of *N. tabacum* are commercially available, have been produced on a large scale by established technologies and used in industry. Tobacco remains one of the economically important crops, and leaf production and processing technology generates significant amounts of available biomass (Dyulgerski, 2020). Several studies elucidated the chemical composition of these tobacco aromatic products (Nedeltcheva-Antonova et al., 2016; Popova et al., 2015; 2018; 2019; 2020a; 2020b). The data in these studies clearly identified the presence of various volatile and semi-volatile compounds reported to be active in bacterial and fungal inhibition processes (alkaloids, terpenoids phenylpropanoids) (Patil et al., 2015; Sharma et al., 2016). In fact, according to some of our previous works, the natural aromatic products used in this study demonstrated antimicrobial activity against some bacterial strains, representing common human skin pathogens being relevant to the development of cosmetic formulations. Promising results were achieved for several *Nicotiana* species as resources for obtaining biologically active extracts and natural aromatic products (Popova et al., 2015; 2017; 2018; 2019; 2020a; 2020b). However, to the best of our knowledge, natural aromatic products from *Nicotiana* spp. have not been studied in plant protection aspects, but the above suggests the potential for their use in this direction.

Therefore, the objective of this work was to assess the biological activity of the natural aromatic products concrete and resinoid obtained from three *Nicotiana* species (*N. tabacum* L.*, N. alata* Link & Otto and *N. rustica* L.), in a direct comparison, against some bacterial pathogens, causing diseases of various crops.

MATERIALS AND METHODS

Plant material

Three *Nicotiana* species (*Solanaceae*) were used as primary plant material for the obtaining of concentrated natural extraction products: *N. tabacum* L., *N. rustica* L., and *N. alata* Link & Otto. *N. tabacum* L. (Common tobacco) is one of the most important cash crops worldwide, cultivated in over 110 countries for the production of various types of tobacco raw materials used by the cigarette industry. In the present study, Oriental-type tobacco of the Plovdiv 7 variety, widely grown in the region of Central Southern Bulgaria, was used. *N. rustica* L. (Aztec tobacco) is also common to a wide geographical zone, although leaf production nowadays is concentrated within a number of countries in Asia, mainly. It is the only other species used for manufactured tobacco products and is one of the most high-alkaloid species of the genus, with nicotine

content as high as 8-10% or more (Sisson & Severson, 1990). *N. alata* Link & Otto (also known as Jasmin tobacco, Persica or Winged tobacco) is a popular ornamental plant worldwide, recognized for its abundant and highly fragrant flowers. In contrast to *N. rustica* and *N. tabacum*, it is one of the most low-alkaloid species of the genus (Sisson & Severson, 1990). Two *N. alata* genotypes were used in the study - one with white flowers and one with pink flowers.

All plants were grown side-by-side on the experimental field of the Tobacco and Tobacco Products Institute, Bulgarian Agricultural Academy, situated in the region of Plovdiv, Central South Bulgaria (42°04'55.2"N 24°42'16.8"E), with humus-carbonate/Rendzina/ soil type (Figure 1a - d). The plant materials were collected during the active growth and flowering of the tobacco plants and were dried. Soil characteristics, production practices and leaf curing technology were described previously (Popova et al., 2017; 2018; 2020a).

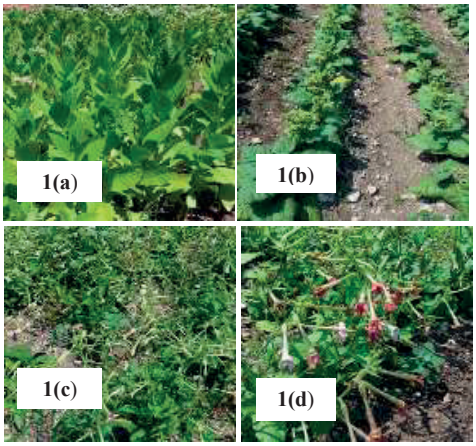


Figure 1. *Nicotiana* plants on the field: 1(a) *N. tabacum* (var. 'Plovdiv 7'); 1(b) *N. rustica*; 1(c) *N. alata* genotype with white flowers; 1(d) *N. alata* genotype with pink flowers (photos by authors)

Natural aromatic products

Two natural aromatic products (Bauer et al., 2001) obtained from the dried leaves of the three *Nicotiana* species, concrete and resinoid, were used in the study. The extraction procedure was performed as described previously (Popova et al., 2017; 2018; 2020a). In brief, concrete was obtained by twofold extraction with petroleum ether (FILLAB, Bulgaria), for 60 and 30 min, respectively, at a temperature of 30ºC and solid-to-liquid ratio of 1:10 (w/v). The solvent was completely evaporated from the combined extracts on a rotary vacuum evaporator at water bath temperature of 35ºC. The yield of concrete (on a dry weight basis) was: *N. tabacum* 1.9 ± 0.07% DW (w/w) (Popova et al., 2018), *N. alata* 1.68% (white flower genotype), 1.35% (pink flower genotype) (Popova et al., 2017) and *N. rustica*, 1.50 ± 0.01% (w/w) (Popova et al., 2020a). Resinoid was obtained by twofold extraction with 95% ethanol for 2.5 h and 2h, at a temperature of 70ºC and solid-to-liquid ratio of 1:10 (w/v). The combined extract was concentrated on a rotary vacuum evaporator, at a temperature of 55ºC. The yield of resinoid was: *N. tabacum*, 20.4 ± 0.19% DW (w/w) (Popova et al., 2018), *N. alata*, 10.27% (white flowers genotype) and 9.50% (pink flowers genotype) (Popova et al., 2017), and *N. rustica*, 15.62 ± 0.09 % (w/w) (Popova et al., 2020a). All extraction products represented semi-solid waxy masses, light to dark brown in color, with specific odor.

Isolation of phytopathogens

The phytopathogens with which this study was conducted are the causes of bacterial diseases on agricultural crops. Four pathogens were isolated and tested. Two of them were isolated from plants with characteristic disease symptoms and two from soil as potential pathogens. Classical methods accepted in phytopathology and microbiology for their initial isolation had been used (Grudeva et al., 2006; Sinclair et al., 2019). The procedure for isolation from plant tissues was as follows: Fresh leaves of infected plants - mulberry (*Morus alba* L.); pepper (*Capsicum annuum* L.) were washed with tap water, the surface was disinfected sequentially for 1 min with sodium hypochlorite (10%), with ethyl alcohol (70%) and washed three times with sterile distilled water. Leaf pieces taken from the boundary between the living and symptomatic tissue of the infectious spots were placed on the surface of Petri dishes with potato-dextrose agar (PDA, Sigma Aldrich Ltd.) and incubated for three days at a temperature 28°C.

Isolation of potentially present phytopathogens in the soil was performed by inoculating of ten-fold dilutions soil suspensions homogenized in sterile saline (0.9% NaCl) on solid nutrient media starch-ammonia agar (SAA). The cultures were incubated under the same conditions as the plant isolates. After incubation from the developed colonies with characteristic morphological features for the presumed pathogens, new cultures were made on the respective nutrient media and series of purification streaks was performed until pure cultures from each isolate were obtained, by Drigalski method (Grudeva et al., 2006). Pure cultures of the isolates were analyzed by macro-morphological characteristics of the colonies and observations under a light microscope, at a magnification of 900x; Gram staining was performed (used: crystal violet as the main dye, lugol's solution as a fixative and 0.5% safranin as the second dye) and a rapid catalase test with H_2O_2 was made.

***In vitro* testing of the biological activity of *Nicotiana* aromatic products**

The biological activity of the aromatic extraction products was determined *in vitro* by the diffusion method in agar wells (Valgas C. et al., 2007). From 24-hour pure cultures of the isolated phytopathogens, suspensions were prepared in 5 ml of sterile saline and incubated at 28°C. Surface inoculations by 24-hour pure cultures were performed on Mueller-Hinton Agar (MHA, Sigma Aldrich Ltd.) and wells (d - 6 mm) were made in the nutrient medium. The density of the bacterial suspensions is approximately 1.5 x 10^8 CFU /ml/ turbidity: 0.5 McFarland - standard/ (Balouiri et al., 2016). Volumes of bacterial inoculums were 0.1 µl on the Petri dish (9 mm).

The concentrated aromatic products, concretes and resinoids were dissolved in 5 ml of 90% ethanol and 5 ml of sterile distilled water is added. The concentration of the tested products in the obtained solutions was 2.5% (w/v). The solutions were prepared immediately before testing. An amount of 100 µL of each extract solution was dropped to the test-wells; the dishes were left for 1 hour at 4°C in order to allow the diffusion of the solution into the media and then incubated for 24h at 37°C. The extract solutions were pre-sterilized with a sterilized through Millipore filter (0.22 mm). The diameter of the sterile zone (mm) was measured. The size of the wells (6 mm) was also included in the presented dimensions of the inhibitory zone. The solution in a ratio of 1:1 of 90% ethanol and sterile distilled water was used as a control. All tests were performed in five reps. Results were presented as mean values ± standard deviation (n = 5).

RESULTS AND DISCUSSIONS

In the present study based on the phenotypic characteristics made, the isolated phytopathogens were identified by genus. The manifestation of the characteristic symptoms of the diseases caused by them, the macro-morphological and microscopic diagnostics were not sufficient for accurately determine of their species affiliation. The results obtained were as follows:

The symptomatic picture of pepper was characteristic of the disease “bacterial scab”. Bacteria have been isolated from infected leaf lesions. The morphology of the colonies allows them to belong to the genus *Xanthomonas*. The colonies were round, with smooth edges, convex, mucoid, creamy-yellow and did not produced fluorescent pigment into the nutrient medium. Bacteria were Gram (-), movable rods; the catalase test was positive. Species of this genus cause a variety of bacteriosis across a wide range of hosts (Figure 2 a - b). Multiple strains, races, and pathovars, depending on the host were differentiated (Jones et al., 2000). Until recently, the main cause of the disease "bacterial scab" on pepper and tomato was considered to be the species *X. campestris* pv. *vesicatoria*, but in recent years its taxonomic status has been revised (Jones et al., 2004). Based on an in-depth study on the species and intraspecific differentiation of bacteria of the genus *Xanthomonas* for Bulgaria, four species were indicated as causative agents of pepper and tomato disease - *X. euvesicatoria*, *X. vesicatoria*, *X. perforans* and *X. gardneri* (Kizheva et al., 2013).

The symptoms of mulberry leaf (spots with yellow halos and necrotic dark-brown spots) as well as the morphological characteristics of the isolate allow the conditional identification of the pathogen as the bacterium *Pseudomonas*

syringae pv. *mori* (Young, 2010). The colonies were round, smooth, with a full edge, convex, pearly whitish or with a pale yellowish tint in the coloration, with weak fluorescence. Microscopic observation showed Gram (-) movable rods. The catalase test performed was positive (Figure 2 c - d). In plant pathology, phytopathogens related to *P. syringae* are classified on the basis of the host into a general phylogenetic group "*P. syringae* - complex", whose taxonomy is controversialy. According to some authors, the group includes about 15 species with more than 60 pathogens (Gomila et al., 2017). According to others, bacterial diseases were caused by an extremely universal and adaptive to hosts and environmental conditions single species (Mansfield et al. 2012; Popović et al., 2021). The species *P. syringae* pv. *mori*, widespread in different parts of the world was considered to be the causative agent of mulberry bacteriosis (Mansfield et al., 2012). It was first registered in 2017-2018 in mulberry plantations in Poland for the conditions of Europe (Krawczyk et al., 2020).

The third bacterial species with which the present study was conducted was isolated from soil and can be attributed to spore-forming bacteria of the genus *Bacillus*. The choice for its initial isolation was made in order to evaluate the biological activity of the tested aromatic products from *Nicotiana* and on representatives of Gram (+) bacteria. The morphological characteristics of the isolated colonies show two bacterial morphotypes, which can be conditionally referred to the group of *B. cereus* - medium-sized, raised colonies, whitish with smooth or slightly curly edges and to the group of *B. subtilis* - relatively large creamy columns with rough texture, with raised wrinkles. Gram staining indicates the presence of G (+) spore-forming rod-shaped bacteria. Both isolates were catalase positive (Figure 2 e - f). Species of this genus were widespread in nature, heterotrophic, with a huge role in the carbon cycle, and have been used successfully in organic farming as part of PGPRB (Beneduzi et al., 2012). At the same time, members of the group *B. cereus* refer to the so-called opportunistic pathogens that cause certain gastrointestinal and skin diseases in humans (Mendes et al., 2013). In the phytopathological aspect, pathogens of *B. subtillis*-complex have been reported, which can cause rot of bulbous plants (Stoyanova et al., 2011).

The bacterial pathogens examined in this study cause serious infectious diseases in a number of crops, leading to deterioration in quality, stability of yields and production, as well as substantial economic losses in the producing countries. Species of the genus *Xantomonas* and the genus *Pseudomonas* are included in the top-10 most important plant pathogenic bacteria (Mansfield et al., 2012).Their ubiquity as part of the epiphytic (plants and seeds) and soil microflora, high virulence and adaptability to various environmental factors, the development of resistance, make the control extremely difficult, mainly with chemicals (Mansfield et al., 2012).

Figure 2. Isolation of bacterial pathogens in the study: 2(a) and 2(b) *Xanthomonas* sp., isolated from spots on pepper leaves; 2(c) and 2(d) *Pseudomonas* sp., from spots on mulberry leaves; 2(e) and 2(f) *Bacillus* sp., isolated from soil - morphotype - 1 (mt-1) defined as *B. cereus* and morphotype - 2 (mt-2) defined as *B. subtillis* (photos by authors)

The results of the *in vitro* application of the aromatic products obtained from the three *Nicotiana* species to the isolated bacterial pathogens showed that all tested natural aromatic products achieved an inhibitory effect on the bacterial pathogens included in the study. Specific differences in activity were observed, depending on the type of product, the species of *Nicotiana* and the species of pathogen. As a general observation, resinoids

were the more effective inhibitors of the four pathogens studied, than the concretes. The strongest inhibition of the gram-negative bacteria (*Xanthomonas* spp. and *Pseudomonas* spp.) was accomplished by the resinoids of *N. rustica* and *N. tabacum*. The zone of inhibition under the influence of these resinoides was over 20.00 mm. The concrete obtained from *N. tabacum* also showed a relatively high inhibitory effect at *Xantomonas* spp. (IZ - 20.00 mm). The effect of the concretes of the other *Nicotiana* species against *Xanthomonas* spp. was lower, in the range of 15.5-13.5 mm. Against *Pseudomonas* spp. the concretes of all four species of *Nicotiana* have a weak effect as the reported inhibitory zone was 11.50-14.50 mm (Figures 3 and 4).

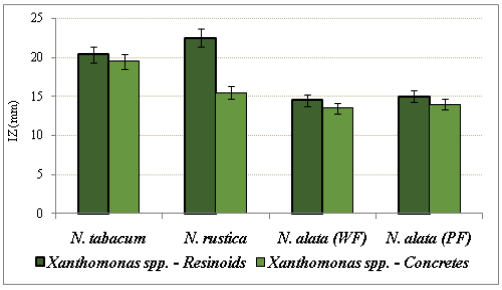


Figure 3. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (-) bacterium *Xanthomonas* spp.

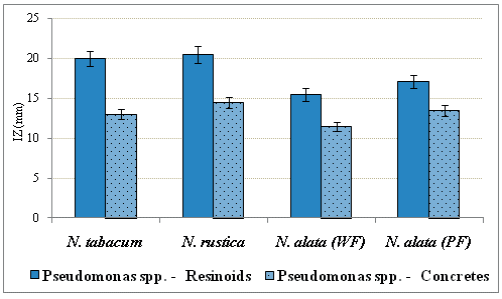


Figure 4. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (-) bacterium *Pseudomonas* spp.

The resinoids of *N. alata* genotype with pink flowers (PF) and *N. rustica* had the highest bactericidal activity against Gram-positive bacteria *Bacillus* spp. At morphotype 1 (mt-1), conditionally defined as *B. cereus* with diameters of the zone of inhibition 24.50 mm and 21.00 mm, respectively, and at morphotype - 2 (mt-2) defined as *B. subtillis* the inhibition zone was 22.50 mm and 20.50 mm. The effect of resinoids from the other *Nicotiana* species was low the diameter of the inhibition zone was less than 20.00 mm (Figures 5 and 6). Bacteria referred to as *Bacillus* spp. were relatively susceptible to *N. rustica* concrete (IZ 19.00 mm and 16.50 mm), also. The bactericidal action of the concretes of the other *Nicotiana* species was low (IZ at mt-1 was 11.50-10.00 mm, and at mt-2 between 8.00-9.50 mm).

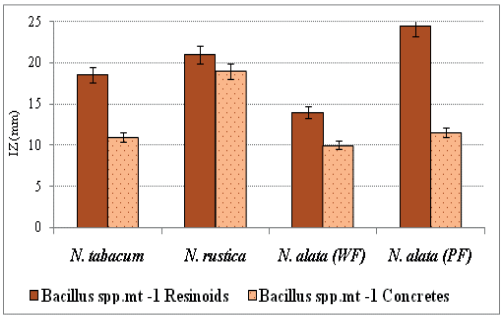


Figure 5. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (+) bacterium *Bacillus* spp. - morphotypes - 1

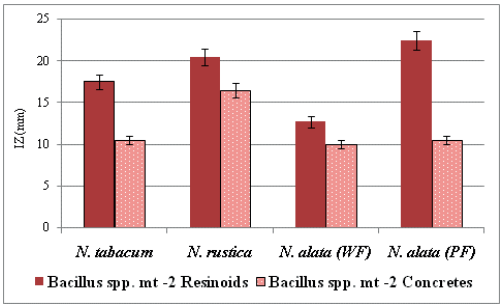
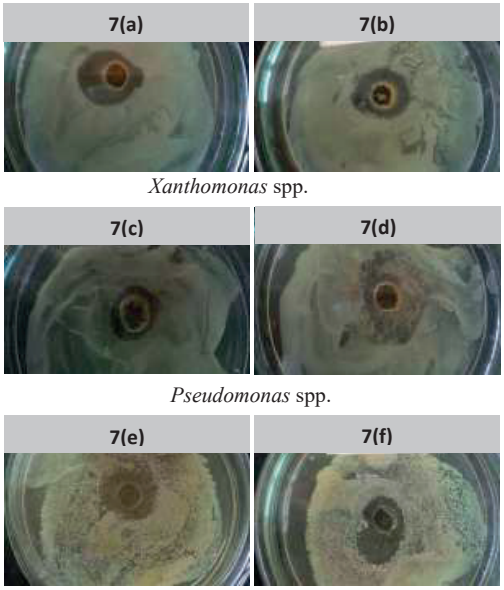


Figure 6. *In vitro* biological activity of *Nicotiana* aromatic products (concretes and resinoids) against the pathogen identified as G (+) bacterium *Bacillus* spp. - morphotypes - 2

The results on the specificity of action of aromatic products depending on the Nicotiana species show that the products with *N. rustica* had the strongest general bactericidal action against both G (-) and G (+) bacteria, followed by the products obtained from *N. tabacum*. The data confirmed the antimicrobial activity of *N. rustica* and *N. tabacum* extracts found by other authors (Bakht, J. & Shafi, 2013). The overall bactericidal activity of the products derived from *N. alata* was less, but differences in activity have been registered between the

two genotypes. Higher activity similar to that reported in *N. tabacum* had the products of the pink genotype with a focus mainly on G (+) bacteria, and those of the white genotype - against G (-) pathogens. The highest inhibition effects achieved in the study are presented on Figure 7.



Xanthomonas spp.

Pseudomonas spp.

Bacillus spp.

Figure 7. Maximal inhibition of bacterial pathogens by *Nicotiana* aromatic products: 7(a) resinoid of *N. tabacum*; 7(b) concrete of *N. tabacum*; 7(c) resinoid of *N. rustica*; 7(d) resinoid of *N. tabacum*; 7(e) resinoid of *N. alata* genotype with pink flowers; 7(f) concrete of *N. rustica* (photos by authors)

The results were consistent with recently published data on the antimicrobial activity of *Nicotiana* species in both medical and phytopathological aspects (Anumudu et al., 2019; Al-Lahham et al., 2020). A number of authors have established the efficacy of *N. tabacum* extracts against human pathogenic bacterial strains of *B. cereus* (Bakht et al., 2013; Patil et al., 2015; Sharma et al., 2016), *P. aeruginosa* (Ameya et al., 2017; Pramono et al., 2018; Al-Lahham et al., 2020). Biological activity of extracts of *N. plumbaginifolia* Viv. against *B. subtilis, B. cereus, B. fusiformis*, and *P. aeruginosa* had been reported (Singh et al., 2010; Kekuda et al., 2017).

Efficacy studies against phytopathogens were fewer in number, but one had been reported on extracts from leaves and roots of *N. tabacum* against *P. solanacearum* and *Xanthomonas axonopodis* pv. *malvacearum*, causative agents of bacteriosis on potatoes and cotton (Singh et al., 2010).

These first results were encouraging, from the perspective of practical use of tobacco aromatic products in phytoprotection, considering the significantly higher yield and better solubility of resinoids, compared to concretes. The results were in full agreement with previous findings about the influence of the used organic solvent on the antimicrobial activity of plant extracts obtained from *Nicotiana* and other species. Typically, extracts obtained with solvents with higher polarity (methanol, ethanol, 1-butanol) demonstrated better biological activities in antibacterial and antifungal tests, compared to those obtained with less polar or non-polar solvents, such as n-hexane (Singh et al., 2010; Patil et al., 2015; Sharma et al., 2016; Ameya et al., 2017; Al-Lahham et al., 2020). The variation patterns between the activities of the two aromatic products were obviously bound to differences in their chemical composition, reflecting not only the effect of solvent polarity, but also that of other extraction factors such as temperature and duration. Therefore, our results were consistent with previously published data about the GC-MS composition of the tested aromatic products (Popova et al., 2017; 2019; 2020a), as well as with the findings about the individual contribution of their major components to the overall biological activity or the synergistic/ antagonistic interactions within the complex mixture (plant essential oils and extracts). Several studies connected the antibacterial, antifungal and antioxidant activities of *Nicotiana* and other plant EOs and extracts with the decisive role of alkaloids, flavonoids, terpenoids, steroids, tannins, saponins, coumarins, and some other classes of chemical compounds or their individual representatives (Patil et al., 2015; Sharma et al., 2016; Nwachukwu, 2017). Therefore, the variation of the antimicrobial activity of the tested concretes and resinoids agreed very well with the different chemical profiles of the two products. Regardless of species, the profiles of *Nicotiana* resinoids, found to be more active in this study, were dominated by nitrogenous compounds, mainly

alkaloids (over 50% of the identified content), followed by diterpenes (over 13%), and phenylpropanoids (over 9%) (Popova et al., 2017; 2019; 2020a); all those represent classes with distinct biological activity as already stated.

In turn, the concretes contained mostly oxygennated aliphatics (over 64% of the identified composition), nitrogenous compounds (about 22%) and oxygenated diterpenes (about 10% share). In general, phenolic and terpenoid compounds were found to be more efficient pathogen inhibitors compared with esters, alcohols and aldehydes; higher activity was attributed to more hydrophobic molecules, such as phenolics and aromatic aldehydes (Raveau et al., 2020); those observations were supported by our results, too. Nicotine, in particular, cited as a key inhibitor, equally effective on G (+) and G (-) bacteria and other pathogens (Bakht et al., 2013), was extracted in high concentrations into the resinoids (by a polar solvent, ethanol), but was a minor compound in the n-hexane extracted concretes. The differences in the antibacterial activity recorded depending on the type of *Nicotiana* in the present study were also in line with the opinions of a number of authors that in addition to nicotine, the individual composition of aromatic products reveals the presence in high concentrations of other potent microbial inhibitors (alkaloid derivatives oxynicotine and cotinine; limonene and others), as well as a number of minor active compounds (e.g., the terpenoids 1,8-cineole, farnesyl acetone and α-pinene) all probably involved in a synergistic pathogen inhibition. The mechanisms of action of these metabolites is not completely cleared yet, but several pathways have been suggested, depending on microorganism cell structure and metabolism specifics Only a few of the studies investigated the direct activity of pure extract components against the respective pathogens (Nazzaro et al., 2017; Pramono et al., 2018; Raveau et al., 2020). Therefore, our results create grounds for further investigation in this aspect, which is set as a possible objective of future research.

CONCLUSIONS

This study examined the biological activity of two natural ready-to-use concentrated aromatic products, resinoid and concrete, derived from the leaves of three species of the genus *Nicotiana* (*N. tabacum, N. rustica* and *N. alata*, in two genotypes) against phytopathogens, causes of bacteriosis in many cultivated plants, fruits and vegetables.

The pathogens with which the tests were performed from the leaves of infected plants (pepper and mulberry) with symptoms characteristic of bacteriosis and from soil were isolated. Based on the macromorphological characteristics of the colonies and microscopic diagnosis of the isolates, they were assigned to the genus *Xantomonas* G (-), to the genus *Pseudomonas* G (-) and two morphotypes to the genus *Bacillus* G (+).

All extraction products were showed inhibitory potential activity against the test pathogens, but there were variations in both species and products. *Nicotiana* resinoids had significantly stronger bactericidal activity (Gram-positive and Gram-negative), compared to the respecttive concretes. The resinoids derived from *N. rustica* and *N. tabacum* had the strongest action against G (-) bacteria defined as *Xantomonas* spp. and *Pseudomonas* spp. The extracts obtained from *N. alata* (genotype with pink flowers) showed the highest bactericidal activity against G (+) bacteria from genus *Bacillus*.

To the best of our knowledge, the aromatic products concrete and resinoid obtained from different *Nicotiana* species have not been studied previously in terms of their phytoprotective potential and these are the first data in this aspect. The outcomes from the study are promising and provide grounds for the development of natural or combined products with bio-protective activity as the next step, as well as for further investigations on the biological activity of *Nicotiana* species.

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