



## Review Article

# Influence of Nicotine Released in Soils to the Growth of Subsequent Maize Crop, Soil Bacteria and Fungi

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## Abstract

Nicotine is a major secondary metabolite of tobacco plant. Nicotine is secreted from the tobacco plant roots, while it is stored in leaf vacuoles. However, some amount of nicotine is released to the soil environment through root exudation. Nicotine released in rhizosphere improves nitrogen, calcium, iron, and zinc uptake; and thus promotes seedlings emergence and vigour, chlorophyll contents and growth of maize as a subsequent crop. However, nicotine also negatively affects multiplication of some beneficial bacteria and fungi, and availability of potassium and phosphorus as well. Therefore, released nicotine may affect the productivity of food crops like maize when cultivated as a subsequent crop to tobacco in the same land. Nonetheless, the fast growing inedible leguminous sun hemp (*Crotalaria juncea* L.) crop can be grown immediately after tobacco harvest before subsequent maize crop, in order to replenish soil fertility and proliferation of beneficial soil bacteria/fungi. The information derived from this review may provide useful insights to smallholder farmers of tobacco and maize crops on the suitability of rotating the two crops with the aim of sustainable intensification. © 2019 Friends Science Publishers

**Keywords:** Allelopathic effect; Cash crop; Cropping systems; Staple cereal crops; Soil microorganisms

## Introduction

Tobacco (*Nicotiana tabacum* L.) is among the leading commercial crops and highly distributed worldwide. The cultivated land is about 3.9 million ha of which 60% is 'flue-cured' tobacco, 13% is burley tobacco, and 12% is oriental (Hu and Lee, 2015; FAOSTAT, 2016a). The top ten producers of tobacco are China, India, Brazil, USA, Indonesia, Zimbabwe, Zambia, Pakistan, Tanzania, and Argentina (Voeks, 2009; Yang, 2010; Marks *et al.*, 2011; FAOSTAT, 2016a). According to Hu and Lee (2015), Africa produced 650,000 tons (8.7%) of the world tobacco leaf in 2012 compared with 440,000 tons (7.3%) in 2003. The famous tobacco growing countries in Africa are Malawi, Tanzania, Zimbabwe, Zambia, and Mozambique (FAO, 2003; Sauer and Abdallah, 2007; Whittington, 2011).

Tobacco is cultivated in different scales depending on the country's target for economic reasons and/or a need to increase family income. Tobacco is mostly grown in rotation with cereals and/or leguminous crops, whereby maize (*Zea mays* L.) is the main food crop (FAOSTAT, 2016b). However, maize productivity in tobacco cultivated systems remained stagnant for the period of five consecutive years from 2012 to 2016 (Fig. 1). This is attributed to the depletion of soil fertility due to high nutrients uptake by tobacco plant, climatic change, and/or usage of unimproved

maize varieties (Denning *et al.*, 2009; MoAFS, 2011; Ngwira *et al.*, 2012). In developing countries, the demand for food crops like maize is increasing due to high population pressure, however area under cultivation of food crops reducing (MoAFS, 2011). Therefore, inclusion of maize in rotations with tobacco in smallholder settings would be one of the sustainable intensification options to enhance food production where tobacco cultivation is inevitable due to its demand as a cash crop.

Rotation mainly improves the soil fertility and sustains its productivity (Butorac *et al.*, 1999; Thierfelder *et al.*, 2013; Shahzad *et al.*, 2016). Rotations of tobacco are found to be compatible with diverse crops such as maize, small grain cereals, grasses, rice (*Oryza sativa* L.), groundnuts (*Arachis hypogaea*), soybeans (*Glycine max* L.), and other legumes (Agrawal *et al.*, 2006; Farooq *et al.*, 2014; Shakeel, 2014; Li *et al.*, 2016; Baek *et al.*, 2017). However, the positive and negative effects associated with tobacco nicotine allelopathy to the subsequent cereal and leguminous crops have not been widely explored (Baek *et al.*, 2017). Few studies have documented allelopathic effects of tobacco nicotine on growth of cereal crops such as maize (Rizvi *et al.*, 1989; Karaman and Brohi, 2013; Farooq *et al.*, 2014; Haq *et al.*, 2018), wheat (*Triticum aestivum* L.) (Shakeel, 2014; Baek *et al.*, 2017) and rice (Shakeel, 2014).

Preliminary studies have indicated that cereal crops are favoured more than the legumes in terms of growth when rotated with tobacco crops (Rizvi *et al.*, 1989, 1999). However to the present, there are three clearly marked contradicting results of tobacco allelopathy on these crops. Firstly, some findings indicate that the growth of both legumes and cereals are hindered by the tobacco allelopathy (Yazdani and Bagheri, 2011; Baek *et al.*, 2017). Some studies highlighted that the growth performance of these crops is equally favoured by the tobacco allelopathy (West and Post, 2002; Reed *et al.*, 2012; Zou *et al.*, 2018a). Thirdly, other studies indicate that cereals growth is more favoured than legumes growth due to tobacco allelopathy (Rizvi *et al.*, 1989, 1999; Farooq *et al.*, 2014). Allelopathy constitutes secondary metabolites released by plants in their roots which in turn affects microorganisms such as viruses, bacteria, and fungi in soils (Narwal *et al.*, 2005). There are very few studies in the tobacco sector addressing the allelopathy effects of nicotine on the growth of subsequent cereal crops such as maize. Therefore, there is a need of establishing studies on allelopathic effects of tobacco nicotine on the productivity of maize when cultivated as a subsequent crop. This will provide a basis for clearly identifying abiotic and biotic factors that affect the productivity of this crop when it is involved in tobacco cultivating systems.

This review focuses mainly on the allelopathic effects of tobacco nicotine on growth of subsequent cereal crop (maize) and the beneficial soil bacteria and fungi. The outcomes of this review would be pertinent to all stakeholders in this sector in understanding the practical implication of tobacco nicotine-crop, soil nutrients and bacteria/fungi interaction.

### Chemical Composition of Tobacco Plant

The constituents of tobacco are not individual compounds but classes of compounds such as alkaloids, proteins (soluble and insoluble fractions), nitrate-nitrogen, amino nitrogen, etc. (Talhout *et al.*, 2011). Nicotine is indicated to be the most abundant of the volatile alkaloids in the tobacco leaf and the high levels of nutrient nitrogen increase nicotine and nitrate levels of the leaf (Leffingwell, 1999). Generally, tobacco plant is chemically composed of sugars, fats and amino acids which are also found in other plants. Other chemical constituents such as aromatic hydrocarbons, phenols, nitrosamines, aldehydes, alkanes, alkynes, toluene, benzene, nitrogen oxide, cadmium, and nicotine are also widely reported (Benowitz *et al.*, 2009; Talhout *et al.*, 2011; Rodgman and Perfetti, 2016). The common chemical composition of tobacco plant is summarised in Table 1. It is widely documented that the biggest portion (96%) of the composition of tobacco metabolites is nicotine (Armstrong *et al.*, 1998; Jacob *et al.*, 1999; Benowitz *et al.*, 2009).

The physical and chemical compositions of tobacco are influenced by the genetics, cropping practices, edaphic factors, climatic conditions, diseases and pests, stalk position, harvesting and curing practices (Leffingwell, 1999). However, there is a dire need of understanding the overriding constituents of tobacco nicotine as it has critical implication on both composition of soil bacteria, fungi and the subsequent crops. In tobacco leaves, various post-harvest reactions during curing degrade nicotine into its nitrogen oxide as well as into cotinine and other alkaloids (Duncan *et al.*, 1991; Wang *et al.*, 2008). Tobacco residues are rich in essential nutrient elements such as calcium (Ca: 3.7%), nitrogen (N: 2.38%), potassium (K: 0.4%) and phosphorus (P: 0.5%) (Table 2); the contents of N and Ca are much higher than the rest nutrients and hence can improve soil fertility or growth of the subsequent crop (Adediran *et al.*, 2004; Chaturvedi *et al.*, 2008; Shakeel, 2014).

### Properties of Nicotine

Nicotine is an organic compound and the main alkaloid found throughout the tobacco plant particularly in leaves (Shoji *et al.*, 2008). Nicotine is a tertiary amine ( $C_{10}H_{14}N_2$ ) consisting of a pyridine and a pyrrolidine ring (Benowitz, 2009), and it forms 2 to 8% of the dry mass of the tobacco leaves (Armstrong *et al.*, 1998). It is water soluble in its base form between 60 and 210°C, having molecular weight of 162.23, melting point of -79°C and boiling point of 247°C (Lide, 2007). Nicotine as a nitrogenous base forms salts with acids that are usually solid and water-soluble (O'Neil, 2006). Fig. 2 presents the structure of nicotine.

### Nicotine Biosynthesis and its Role in Tobacco Plant

Nicotine biosynthesis in tobacco plant starts from the prominent components, amino acids, aspartic acid, ornithine and methionine (Leete, 1992; Dewick, 2002). These amino acids together with a glucose degraded compound namely glyceraldehydes (Fig. 3) construct a pyridine and pyrrolidine which eventually combines them under the influence of the plant's jasmonic acid to produce nicotine in the tobacco plant roots with heterocyclic pyridine and pyrrolidine rings (Dewey and Xie, 2013).

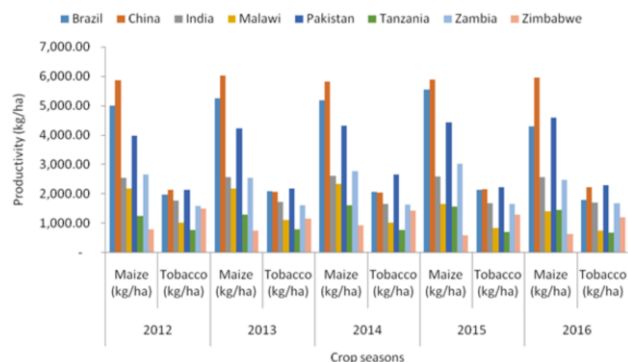
Jasmonic acid at the root zone has an influence in regulating expression of gene as well as in stimulating synthesis of the enzymes required for nicotine synthesis (Steppuhn *et al.*, 2004; Katoh *et al.*, 2005). The pyridine rings formed as a results of series of reactions through ornithine decarboxylase (ODC), *putrescine N-methyltransferase* (*pmt*) and *N-methylputrescine oxidase* (*MPO*) that are responsible for nicotine synthesis in tobacco plant by over 95% (Steppuhn *et al.*, 2004; Katoh *et al.*, 2007; DeBoer *et al.*, 2011). Regulation of root growth and biosynthesis of nicotine is mediated by Nicotine Uptake Permease1 (NUP1), localized at the root plasma membranes (Kato *et al.*, 2015). Synthesized nicotine is then transported

**Table 1:** Chemical constituents of tobacco plant (Adapted from Down, 2014)

Chemical name	Chemical formula
Benzo[a]pyrene	C <sub>20</sub> H <sub>12</sub>
Nicotine-derived nitrosamine ketone (NNK)	C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>
N-Nitrosornicotine (NNN)	C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> O
Nornicotine	C <sub>9</sub> H <sub>12</sub> N <sub>2</sub>
Diacetyl	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>
Anatabine	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub>
Maltol	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
Myosmine	C <sub>9</sub> H <sub>10</sub> N <sub>2</sub>

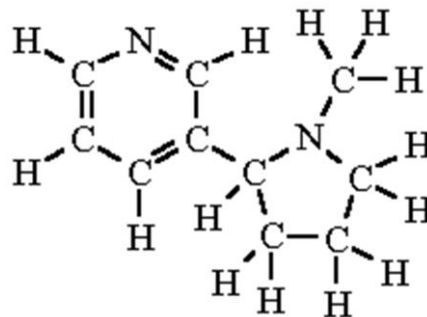
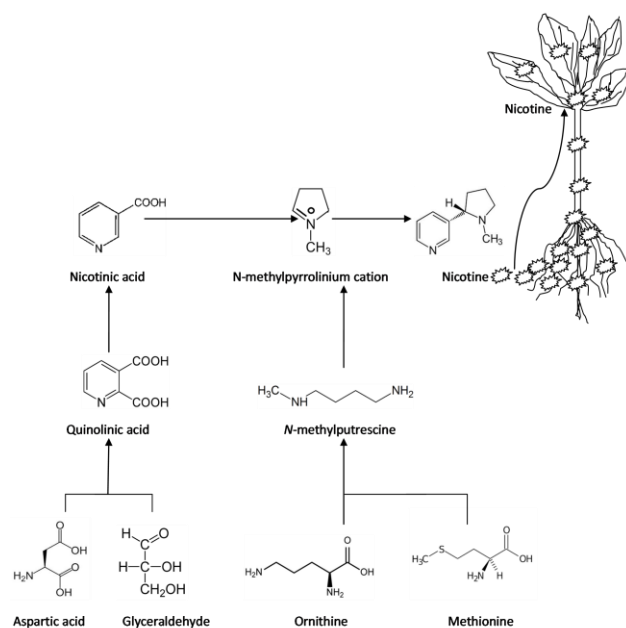
**Table 2:** Physio-chemical composition of most tobacco residues (Adapted from Shakeel, 2014)

Evaluate variables	Values
Moisture content	7.70%
pH	5.6–6.0
Ash	35.4% (dry)
Total nitrogen (Kjeldahl method)	2.38% (dry)
Carbon/Nitrogen ratio	13.5–15.9
Phosphorus	0.5% (dry)
Calcium	3.7% (dry)
Magnesium	0.55% (dry)
Potassium	0.4% (dry)
Nicotine	1.50% (dry)

**Fig. 1:** Tobacco and maize production trend for Brazil, China, India, Malawi, Pakistan, Tanzania, Zambia and Zimbabwe (FAOSTAT, 2016ab). This presentation depicts a general stagnant trend of maize productivity in these selected countries where tobacco is also cultivated

through xylem from the roots to the leaves, where it accumulates (Shoji *et al.*, 2008) in the leaf vacuoles (Shitan *et al.*, 2009). Genetically, the contents of nicotine in tobacco plants is thoroughly controlled by two prominent distinct loci *NICOTINE 1* and *NICOTINE 2* (*NIC1* and *NIC2*) (Hibi *et al.*, 1994).

The normal agronomic practice of topping flower parts prior to harvesting of ripened leaves has the desirable increase of leaf mass. However, this practice also has the influence of increasing nicotine in leaves (Xi *et al.*, 2005; Shi *et al.*, 2006; Wang *et al.*, 2008). The increase in nicotine is linked to the lessening of auxins flowing from apex down to the roots where nicotine is synthesized. The effect of removing flower parts also results into an increase in

**Fig. 2:** Nicotine (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>) structural formula. The edges where hydrogen (H) is bonded allow its replacement by other cations such as Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>2+</sup>, micronutrients (Cu<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup> or Fe<sup>3+</sup>, Zn<sup>2+</sup>), phosphate ions (PO<sub>4</sub><sup>3-</sup>, HPO<sub>4</sub><sup>2-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>)**Fig. 3:** Model of nicotine synthesis in tobacco plant. This shows the prominent components in which amino acids become the initiator of nicotine synthesis in the roots and then transported to the leaves where nicotine is stored in vacuoles.

jasmonic acid concentrations to the shoots and leaves within a short period (Shi *et al.*, 2006). The produced nicotine has various functions to tobacco plant such as defence against predators and in triggering the formation of linolenic acid and jasmonic acids, the compounds that aid in plant growth processes (Ballaré, 2011).

### Nicotine Pathways to the Soil Environment as Allelopathy and Allelochemicals

The major nicotine pathway to the soil environment is through root exudation although the decomposing tobacco roots in the soils may also be accounted as the minor

**Table 3:** Overview on the effect of tobacco nicotine allelopathy on different crops, microorganisms and soil properties

Crops in rotation	Effect of tobacco residues/allelopathy on crop growth	Effect on Soil properties	Effect on Microbial population	References
Maize, Mung bean, Lettuce and wheat crops		Improvement in: electrical conductivity; water intake and its holding capacity; increase in N, Mg, Zn, Fe, nicotine and total phenolics; increase in soil pH; total salts stability	Unfit for the insects breeding; reduce in the ants <i>Lasiusnigernesi</i> in the gardens; affects survival and/or proliferation of poor biodegradable microbes; may promote growth of plants' mutualistic fungi	Aggelides and Londra (2000); Bulluck <i>et al.</i> (2002); Agrawal <i>et al.</i> (2006); Candemir <i>et al.</i> (2012); Cercioğlu <i>et al.</i> (2012); Farooq <i>et al.</i> (2014)
Maize crop	Increase in: stand establishment; leaf emergence; growth; dry matter yields; total N concentrations; chlorophyll content			Rizvi <i>et al.</i> (1989); Karaman and Brohi (2013); Farooq <i>et al.</i> (2014); Haq <i>et al.</i> (2018); Aggelides and Londra (2000); Bulluck <i>et al.</i> (2002); Agrawal <i>et al.</i> (2006); Candemir <i>et al.</i> (2012); Cercioğlu <i>et al.</i> (2012); Dakora and Phillips (2002); Lind (2006); Shakeel (2014)
Rice crop	Protection against snail problem			Shakeel (2014)
Wheat crop	Increase in N; decrease in germination rate			Shakeel (2014); Baek <i>et al.</i> (2017)
Vegetables	Increase in N			Shakeel (2014)
Cowpea crop	Improvement in growth and yield			Agrawal <i>et al.</i> (2006)
Mungbean	Reduction in: emergence uniformity, seedling dry weight and chlorophyll contents			Farooq <i>et al.</i> (2014)

pathway (Darwent *et al.*, 2003). Following these pathways, tobacco plant has strong allelopathic effects, by secreting allelochemicals, to the subsequent crops (Dennis *et al.*, 2010; Cheng and Cheng, 2016). Therefore, tobacco plant has allelopathic effects to the subsequent crops because of its nicotine effects produced as a secondary metabolites towards the productivity of other plants and the composition of soil bacteria and fungi in natural communities and agricultural systems (Einhellig, 1995).

On the other hand, tobacco plant produces non-nutritive allelochemicals as secondary metabolites which are also active media of allelopathy. These allelochemicals released by tobacco plants include amino acids and aspartic acids (Leete, 1992; Dewick, 2002), hydrocarbons, phenols, alkanes, alkynes (Benowitz *et al.*, 2009; Talhout *et al.*, 2011), flavonoids, alkaloids and isoprenoids (Nugroho and Verpoorte, 2002). All these chemicals could also have effects to the subsequent crops even though they exist in small concentrations compared with the nicotine.

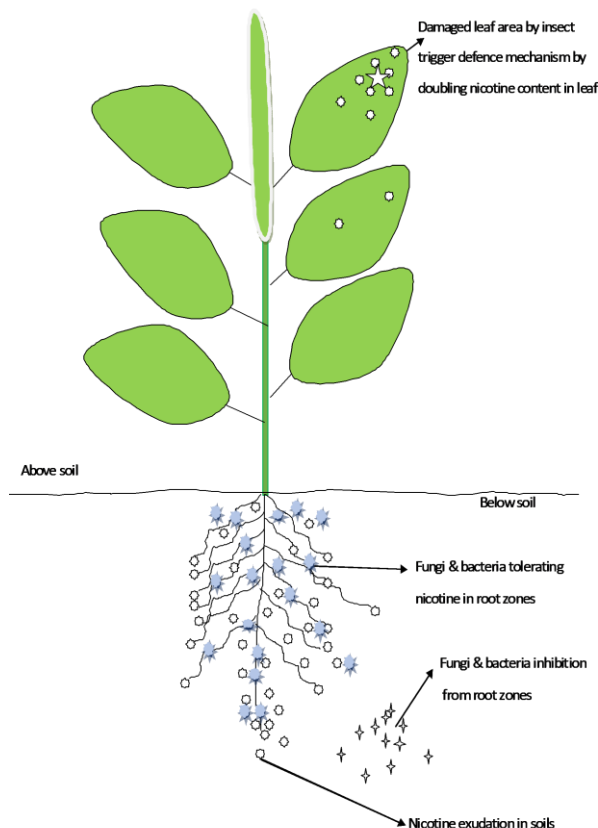
### Nicotine: as Defence Agent against Herbivores and Soil Nutrients Competitors

Any wound caused by herbivores on part of the tobacco leaf stimulates synthesis of jasmonic acid, the hormone which is distributed throughout the tobacco plant (Ballaré, 2011). The same hormone is immediately transported through phloem to the roots which is the important site for nicotine synthesis (Baldwin and Ohnmeiss, 1994; Hibi *et al.*, 1994;

Zhang and Baldwin, 1997). Jasmonic acid at the root zone is involved in the regulation of gene *pmt* for nicotine synthesis and nicotine is transported via xylem to the leaves where its contents double in damaged leaf (Fig. 4; Steppuhn *et al.*, 2004; Katoh *et al.*, 2005; Shoji *et al.*, 2008).

The same defence characteristic of nicotine also happens in the root zones where it is released to the soil environment through root exudation and residual roots decomposition (Ndakidemi and Dakora, 2003). Nicotine released passively at meristematic root regions to the soil rhizosphere plays a key role in protecting the plant against major groups of soil bacteria and fungi hence reducing competition for soil nutrients which could have been metabolized by these pathogens (Darwent *et al.*, 2003; Walker *et al.*, 2003; Adediran *et al.*, 2004; Niu *et al.*, 2016). Based on these scenarios, tobacco seems to be a unique crop probably in the world for its defensive mechanisms against predators, biota above and below the soil surface, respectively (Fig. 4). Evaluating these mechanisms in field conditions where productivity of crops cultivated subsequent to tobacco and the composition characteristics of the soil bacteria/fungi is important under diverse agro-settings.

Regarding the damages caused by the excessive allelopathic effects of tobacco nicotine, other research paths are explored worldwide, such as the use of microorganism normally called Plant Growth Promoting Rhizobacteria (PGPR) (Saharan and Nehra, 2011; Gholami *et al.*, 2012). PGPR are free-living soil-borne bacteria that colonize the rhizosphere and have great importance in governing the



**Fig. 4:** Nicotine acts in defence mechanism against predators and soil bacteria and fungi. This Figure describes the zones at which the released nicotine into soils by tobacco roots suppresses fungi and bacteria. Only fungi and bacteria that are tolerant to high nicotine levels persist in the root zones and hence reduce competition for soil nutrients (Darwent *et al.*, 2003; Ballaré, 2011; Niu *et al.*, 2016)

functional property of terrestrial ecosystems and have important role in plant health and soil fertility (Gholami, 2011). The famous known species of PGPR belong to the genus *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacte*, *Burkholderia*, *Bacillus* and *Serratia* (Yazdani *et al.*, 2009; Saharan and Nehra, 2011). Karnwal (2012) isolated *Pseudomonas*, *Bacillus*, *Azospirillum*, and *Azotobacter* which were asserted to be useful as crop-enhancer and bio-fertilizer for production of cereals like maize. Gholami *et al.* (2012) screening for PGPR properties showed significant difference between indole-3-acetic acid (IAA) and siderophores production and phosphosolubilization between *Pseudomonas* spp. and *Bacillus* spp. but *Pseudomonas* was a better producer of hydrogen cyanide (HCN) and siderophores. Therefore, understanding of the implications of nicotine as a defence agent against predating herbivores as well as favouring solubility and availability of essential nutrients in soils relative to other crops growing with/after tobacco is inevitably important.

### Nicotine Retention to Acidic and Alkaline Soils

Nicotine an alkaloid having two N atoms, one in the pyridine and the second in the pyrrolidine ring released to the soil by the tobacco plant may form salts in acidic soils (Leffingwell, 2001). However, the formed salts cannot be easily crystallized and are readily soluble in water (Talhout *et al.*, 2011) or may be retained in the interlayer and inter-lattice positions of the soils due to its nature of donating electron through aliphatic N of its pyrrolidine ring (Singhal and Singh, 1974; Graton *et al.*, 2003). In clay soils, adsorption of nicotine at low concentrations is through formation of hydrogen bonds, while at higher nicotine concentrations is through electron-donor-acceptor interactions (Singhal and Singh, 1974; Graton *et al.*, 2003).

Under acidic conditions, nicotine is adsorbed strongly through protonation of the pyrrolidine N atom by receiving a  $H^+$  (proton) from carboxylic groups of the humic acid to form nicotine-humic acid salt (Khairy *et al.*, 1990; Golia *et al.*, 2007; Xue *et al.*, 2008). In alkaline soils, nicotine is not strongly adsorbed due to the pair of electrons from pyrrolidine N atom of nicotine being quickly transferred to the humic particles and similarly to the electrons on the pyridine N atom (Khairy *et al.*, 1990). Therefore, nicotine is adsorbed more in acidic than alkaline soils and does not require much temperature for its adsorption (Rakić *et al.*, 2010).

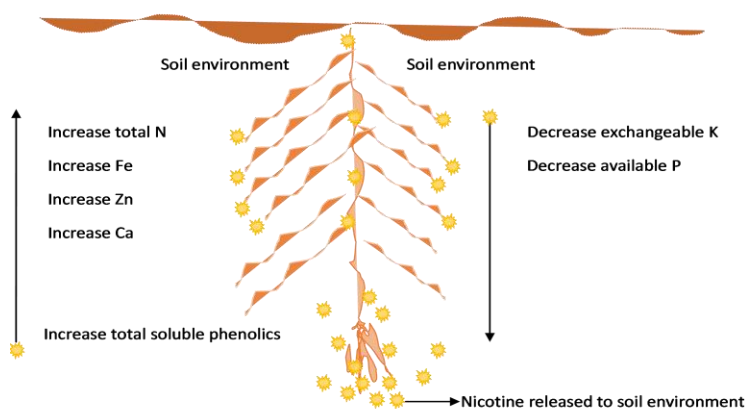
The nicotine adsorbed in soil colloids has residual effect on growth of plants grown on such soils as well as the survival and proliferation of beneficial soil bacteria and fungi (Adediran *et al.*, 2004). Residues of tobacco nicotine in soils also increase the total soluble phenolics, which may have both positive and negative effects to the subsequent crop and the beneficial soil bacteria and fungi (Weidner *et al.*, 2005; Farooq *et al.*, 2014). Many studies have documented the implication of soil reaction (acidity and/or alkalinity) on the adsorption of nicotine by soils. However, similar literature does not critically consider contribution of soil texture and nicotine-organic carbon and/or micronutrients such as copper (Cu), Fe, manganese (Mn), and Zn interactions. There is still a gap of understanding these interactions and the period by which nicotine persists in soil colloids and its associated effects under alkaline and acidic conditions.

### Allelopathic Effects of Tobacco Nicotine on Maize Growth

Tobacco plant through its roots release nicotine to the soil environment is considered to be beneficial on its survival because it reduces nutrients competition against other plants, soil bacteria and fungi (Darwent *et al.*, 2003; Walker *et al.*, 2003; Batish *et al.*, 2008). Very few research have been conducted to study the allelopathic effects of tobacco (nicotine) on established and growth of cereal crops in fields (Kruse *et al.*, 2000; Farooq *et al.*, 2014; Baek *et al.*, 2017).

**Table 4:** Selected nicotine-degrading microorganisms

Microorganism	Time (h or d)	Required conditions (pH & Temp)	Nicotine degradation efficacy (%)	Reference
<b>Gram-negative bacteria</b>				
<i>Acinetobacter</i> spp. TW	12 h	7.0 & 30°C	94.70	Wang <i>et al.</i> (2011)
<i>Ochrobactrum</i> spp. 4-40	12 h	7.0 & 28°C	51.50	Ma <i>et al.</i> (2012)
<i>Pseudoxanthomonas</i> spp. 5-52	12 h	7.0 & 28°C	47.20	Ma <i>et al.</i> (2012)
<i>Sinorhizobium</i> spp. 5-28	12 h	7.0 & 28°C	72.50	Ma <i>et al.</i> (2012)
<i>Agrobacterium tumefaciens</i> S33	18 h	7.0 & 30°C	98.87	Wang <i>et al.</i> (2009)
<i>Sphingomonas</i> spp. TY	18 h	7.0 & 30°C	98.70	Wang <i>et al.</i> (2011)
<i>Pseudomonas</i> spp. CS3	24 h	7.0 & 30°C	98.6	Wang <i>et al.</i> (2012)
<i>Pseudomonas</i> spp. HF-1	25 h	6.5-7.5 & 30°C	99.6	Ruan <i>et al.</i> (2005)
<i>Ochrobactrum intermedium</i> DN2	36 h	7.0 & 30°C	97.60	Yuan <i>et al.</i> (2006)
<b>Fungi</b>				
<i>Aspergillus oryzae</i> 112822	40 h	6.5 & 28°C	60.80	Meng <i>et al.</i> (2010)
<b>Gram-positive bacteria</b>				
<i>Arthrobacter</i> spp. HF-2	48 h	7.0 & 30°C	94.20	Ruan <i>et al.</i> (2006)
<b>Gram-negative bacteria</b>				
<i>Pseudomonas</i> spp. Nic22	48 h	6.5 & 30-34°C	96.50	Chen <i>et al.</i> (2008)
<i>Rhodococcus</i> spp. Y22	52 h	7.0 & 28°C	96.00	Gong <i>et al.</i> (2009)
<b>Fungi</b>				
<i>Cunninghamella echinulata</i> IFO-4444	13 d	5.5 & 28°C	72.00	Uchida <i>et al.</i> (1983)
<i>Pellicularia filamentosa</i> JTS-208	20 d	5.5 & 28°C	09.00	Uchida <i>et al.</i> (1983)



**Fig. 5:** Effects of nicotine released to the soil on nutrients and total soluble phenolics. The presentation pinpoints that nicotine in soils tends to create favourable environment for increasing N, Fe, Zn, Ca and total soluble phenolics. However, the negative effect of nicotine is towards decreasing exchangeable K and available P in the soil

Tobacco allelopathy may reduce or increase growth of subsequent crops, because the soil still may contain remnants of nicotine as released to the soil (Wu *et al.*, 2001). Nicotine has been associated with the increasing chlorophyll content, leaf weight, seedling length and radicle length in cereal crops (Rizvi *et al.*, 1989, 1999; Farooq *et al.*, 2014). Other studies indicated that germination of grain legumes such as mug bean, soybean and cereals (red fife wheat) was hindered by the allelopathic chemicals released by the tobacco plants when sown in rotations (Yazdani and Bagheri, 2011; Baek *et al.*, 2017). Some studies have shown rotation benefits of tobacco with cereals such as maize and legumes (West

and Post, 2002; Reed *et al.*, 2012; Li *et al.*, 2016; Zou *et al.*, 2018b). However, majority of studies have indicated beneficial effects of maize growth when rotated with tobacco (Mamolos and Kalburtji, 2001; Yin *et al.*, 2009; Farooq *et al.*, 2014; Zhou *et al.*, 2014; Kim *et al.*, 2017). Table 3 summarizes the allelopathic effects of tobacco nicotine on various components of the ecosystem, including maize crop among others.

Nicotine released into the rhizosphere in sandy loam soils have been attributed to the substantial increase in growth rate, chlorophyll, number of leaves, plant height and dry matter yields in subsequent cereals (Farooq *et al.*, 2014). However, in silty loam soils,



Yazdani (2014) indicated that nicotine allelopathy on maize decreased seedlings emergency rate, seedling weight, vigour and chlorophyll content. Allelopathic effects of tobacco on growth of cereals are generally positive but there are some few cases of negativity. Allelopathic effects may differ with soil types and/or with varieties of crops used (Farooq *et al.*, 2014; Yazdani, 2014). Studies about effects of tobacco nicotine on the subsequent crops under different soil types are limited. This prompts a need for execution of further studies in order to address effects of tobacco nicotine released into soils to such cropping systems.

Tobacco nicotine has strong allelopathic growth beneficial effects to the subsequent cereals compared with other crops such as grain legumes (Fig. 5; Farooq *et al.*, 2014). This could be due to genetic variability between cereals and legumes and higher susceptibility of legumes to certain disorder in response to nicotine exposure. These genetic variability benefits for cereal crops such as maize could also be associated with increased uptake of total N, Fe, Zn and Ca (López-Lefebvre *et al.*, 2001, 2002; Farooq *et al.*, 2014; Zou *et al.*, 2018b). The increased total N in soils could be due to the suppressing effect of nicotine on soil bacteria such as nitrosomonas, nitrococcus and nitrobacter involved in converting ammonia into nitrate (usable form by plants) and hence increase total N in soils (Farooq *et al.*, 2014). This process also contributes to the minimization of soil N losses (Jabran *et al.*, 2012), that could be beneficial for both cereal and legume crops. Minimization of N loss in the soil causes an increase in total N, which has a great influence on boosting growth of maize crop.

Nevertheless, other studies have revealed that nicotine allelopathy on its genetic nature influenced the tobacco plant. This effect is recognized when tobacco takes up more exchangeable K and available P. This situation leads to decreasing concentrations of P and K nutrients in soils (Xu *et al.*, 2008; Farooq *et al.*, 2014; Moula *et al.*, 2018). Deficiencies of K and P in soils are inevitably likely to negatively affect performance of the subsequent cereal crops (Aziz *et al.*, 2010; Anees *et al.*, 2016; Pavuluri *et al.*, 2017). Nutrient K plays a positive role on transfer of N, starch, sugar, fat and protein synthesis (Rostami, 1997). On the other hand, P is responsible in growth of dense roots for nutrients absorption, seed and/or fruit formation and stem strength (Zhu and Lynch, 2004).

Very few researches on the effects of tobacco nicotine on soil nutrients have been conducted. Therefore, further research is required to study if cereals/legumes used as subsequent crops have abilities of taking up nicotine from the soils and trace associated effects to these crops.

### **Allelopathy Effects of Tobacco Nicotine on Soil Bacteria and Fungi**

Plants deposit their photosynthetically fixed carbon into their direct surroundings such as spermosphere,

phyllosphere, rhizosphere and mycorrhizosphere while feeding the microbial community and influencing their composition and activities (Mendes *et al.*, 2013). Some fungi and bacteria in soils cause a range of plant diseases and in some cases to devastate agricultural crops while others provide resistance to plant pathogens (Marschner *et al.*, 2001). The same soil organisms decompose plant residues, provide nutrients to plants, and stimulate plant growth (Jarosz and Davelos, 1995; Marschner *et al.*, 2001; Smalla *et al.*, 2001). Knowledge of the diversity and structure of bacterial and fungal communities in bulk and rhizosphere soils can lead to a better understanding of their roles in soil ecosystems. The rhizospheres of young maize plants are associated with Ascomycetes order Pleosporales, while different members of the Ascomycetes and basidiomycetic fungi are detected in the rhizospheres of senescent maize plants (Gomes *et al.*, 2003). Maize growth stages influence density, diversity and community structure of some bacterial and fungal groups present in its rhizosphere (Cavaglieri *et al.*, 2009).

Bacteroidetes and Cyanobacteria are the genes mostly enriched in the maize rhizosphere as the rhizosphere niche supports greater functional diversity in catabolic pathways (Li *et al.*, 2014). These genes in maize rhizosphere are involved in carbon (C) fixation and degradation especially for hemicelluloses, aromatics and lignin. They are also responsible in N fixation, ammonification, denitrification, polyphosphate biosynthesis and degradation, sulphur (S) reduction and oxidation (Li *et al.*, 2014). Therefore, maize rhizosphere is a hotspot of Bacteroidetes and Cyanobacteria genes. They mostly originate from dominant soil microbial groups such as Proteobacteria, providing functional capacity for the transformation of labile and recalcitrant organic C, N, P and S compounds (Li *et al.*, 2014).

There are significant growth-related dynamic changes in bacterial community structure associated with the phylum *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* mainly the genera *Burkholderia*, *Flavisolibacter* and *Pseudomonas*. It was found that different growth stages affected the bacterial community composition in maize soil (Yang *et al.*, 2017). According to Yang *et al.* (2017), there are some unique genera in maize rhizosphere such as *Nonomuraea*, *Thiobacillus*, and *Bradyrhizobium*. These are beneficial for the plant growth indicating that the selectivity of root to rhizosphere microbial is an important mechanism leading to the differences in the bacteria community structure between rhizosphere and the bulk soil (Yang *et al.*, 2017). Aira *et al.* (2010) found that older maize plants did not promote higher biomass or microbial growth rates suggesting complex interactions between maize plants and rhizosphere microbial communities. This observation was attributed to the differences in the quality and/or composition of root exudates. The bacterial and fungal community shifts in response to maize rhizosphere are also related to the changes in root exudation patterns (Demathis *et al.*, 2012).

Regardless of their composition in soils, other factors

such as allelopathy may have significant effect on the bacteria population. Tobacco nicotine allelopathy has a depressing effect on composition of soil bacteria, fungi and their activities (Adediran et al., 2004). The population of soil bacteria and fungi decreased significantly when tobacco were planted continuously compared with when it was rotated with maize (Niu et al., 2016). This suggests that the decrease in soil bacteria and fungi could be due to the nicotine released to the soil environment by the tobacco plant roots.

Despite suppression effect of nicotine on soil bacteria and fungi population, still there are few bacteria in soils (Fig. 4) such as *Pseudomonas* which have great ability to withstand nicotine toxicity (Table 4). These bacteria withstand high nicotine levels under the pH levels ranging from 6.5–7.0 (Wang et al., 2012). *Pseudomonas* (gram-negative) strain CS3, Nic22, ZUTSKD were found to tolerate high nicotine concentration up to 5 g L<sup>-1</sup> in soil with high efficacy (over 85.4%) in degrading nicotine in soil at 30–34°C and pH range of 6.0–7.0 (Chen et al., 2008; Zhong et al., 2010; Wang et al., 2012). Strain HF-1 identified from the genus *Pseudomonas* (gram-negative) was found to have higher efficacy of nicotine-degrading by 99.6% at the soil pH range of 6.5–7.5 (Ruan et al., 2005).

At the soil pH of 4–10, gram-negative bacteria genera *Acinetobacter* spp. TW and *Sphingomonas* spp. TY were observed to have greater efficacy to degrade 1 g L<sup>-1</sup> of nicotine by 94.7% and 98.7% within 12 – 18 hours, respectively at temperatures ranges of 15–45°C (Wang et al., 2011). However, strain TW was found to have greater tolerance of high nicotine of up to 4.44 g L<sup>-1</sup>. The strain S33 which was classified as *Agrobacterium tumefaciens* is among of the few bacteria identified to have higher tolerance ranges of nicotine (0.5–5 g L<sup>-1</sup>) with 98.87% efficacy of degrading nicotine, but at only pH level of 7.0 and temperature of 30°C (Wang et al., 2009). The only Gram-positive bacteria *Arthrobacter* spp. HF-2 was observed to have maximum degradation of soil nicotine by 100% at level of pH 7.0 and temperature of 30°C but with very low nicotine tolerance level of up to 0.7 g L<sup>-1</sup> (Ruan et al., 2006). Therefore, gram-positive bacteria seem to have very low tolerance degree to nicotine in soils than gram-negative bacteria.

Fungi groups generally have low efficacy in degrading nicotine compared with the bacteria. For instance, Basidiomycetes and Saprophytes such as *Pellicularia filamentosa* JTS-208 and *Cunningham ellaechinulata* IFO-4444, respectively, have less abilities to degrade (S)-nicotine (Uchida et al., 1983). *P. Filamentosa* was observed to degrade nicotine by 9% into nornicotine in 20 days, whereby *C. echinulate* degrade nicotine by 72% into nornicotine and N-methylmyosmine within 13 days (Uchida et al., 1983). Fungus *Aspergillus oryzae* designated as strain 112822 was observed to bio-degrade nicotine by 60.8% in 40 h at pH level of 6.5 and temperature of 28°C (Meng et al., 2010). In general, fungi are

considered to have poor abilities in tolerating and degrading high nicotine levels in the ecosystems.

In summary, most of the isolated bacteria that degrade-nicotine have been largely explored in China and partly in India. Studies on nicotine degrading bacteria are limited in most of countries producing tobacco. Majority of tobacco producing countries need also to engage more on research pertaining to the isolation of nicotine degrading bacteria and fungi in soils since share of tobacco produced increased from 57% in 1961 to 90% in the year 2006 (Geist et al., 2009). The strains tolerating high efficacy levels of nicotine in soils with good abilities of degrading nicotine can eventually be used for bioremediation of nicotine contaminated soils among the main tobacco production and industrial areas.

### Management Options for Residual Effects of Nicotine in Tobacco Production Areas

The protection mechanisms possessed by tobacco plants through its nicotine against predators and in gaining competitive advantage on nutrients over other plants and/or microorganisms retain a good trait for tobacco survival (Ballaré, 2011). Nicotine synthesized in tobacco plant also potentially threatens the performance of subsequent crops by inhibiting the rhizospheric acquisition as well as uptake of some macronutrients such as exchangeable K and available P. With this in mind, tobacco crop may be grown in rotation with screened plants/crops that have abilities to withstand the residual effects of nicotine or are able to restore soil fertility such as sunn hemp (*Crotalaria juncea* L.) plant (Márton, 2010). Sunn hemp is a fiber inedible leguminous crop characterized by low N requirements due to its ability to fix atmospheric N, grows in marginal soils, drought resistance and resistance to root-knot nematodes (Cook and White, 1996). The fastest growing species of the *C. juncea* plants may be used also as part of a cropping system for integrated pest management (Tavares et al., 2011).

*Crotalaria* plant may be used to intercede the tobacco's and food crops' main seasons and it is expected to create conducive environments for the subsequent food crop in the same piece of land after tobacco plant has been harvested. A staple cereal crop such as maize can then be grown in the next main season in order to take advantage of replenished soil fertility, probably with also abundance of beneficial soil bacteria. However, in situations where land is scarce, there is also a need of ensuring that the supply of macronutrients such as K and P does not confront the growth and/or productivity of subsequent food crops. Optimization and sustainable productivity of cereal crops and improvement of food security in tobacco producing areas could be met by continuously use of nutrients K and P among other essential nutrients as well as maintaining optimal levels of other soil properties.



The mechanisms in creating competitive advantage of tobacco plant against soil bacteria and fungi for nutrients in soils have also remained poorly understood. Therefore, use of both molecular/genetic approaches and ecological/environmental techniques such as allelopathy may be important in evaluating the most appropriate options in management of nicotine discharged and adsorbed into soils in tobacco producing areas. This aims at optimizing growth and productivity of food crops cultivated in rotations with tobacco but along with enhancing diversity of soil bacteria and fungi.

## Conclusion

Tobacco is a unique crop for its defensive mechanisms against predators, bacteria and fungi above and below the soil ground, respectively. Tobacco nicotine allelopathy favours growth of subsequent food cereal crops such as maize as it enhances availability of essential nutrients such as total N, Ca, Fe and Zn in soils. However, the same nicotine decreases availability of K and P, which may have adverse effects on the overall growth and productivity of subsequent crops if these nutrients are not supplemented in soils. Therefore, in future there is a need for extending research on allelopathic effects of tobacco towards productivity of cereals standing crop such as maize. Tobacco nicotine allelopathy also decreases significantly the population of bacteria and fungi in soils when tobacco is continuously cultivated instead of being rotated with crops of different species such as food cereal crops. In addition, our suggestion is that inedible leguminous plants such as *Crotalaria* may be planted in same field immediately after tobacco harvest. In this way, the subsequent food cereal like maize will benefit from the replenished soil fertility and improved structure as well as the restored abundance of beneficial bacteria.

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