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GROWTH PARTICULARITIES OF AMERICAN POKEWEED – PLANT WITH MULTIPURPOSE UTILIZATION

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ABSTRACT

American pokeweed (*Phytolacca americana* L.) is a good ornamental plant due to its ability to grow rapidly, broad and tall vegetation, and attractive grape-like fruits. However, American pokeweed and some useful chemical constituents, which accumulate in different parts of this plant, until present, in our opinion, are not utilised sufficient effectively. The leaves and seeds of *Phytolacca americana* L. produce pokeweed antiviral protein with increased antiviral and antifungal activities. Natural dyes accumulated in fruits can be used in the food, cosmetic, pharmaceutical and textile industries. The purpose of this work was to study the growth particularities of pokeweed plants propagated by seeds, and to establish the content and antioxidant activity of main constituents (polyphenols and colorant substances) in ripe fruits. American pokeweed demonstrated the excellent adaptivity of seeds and seedlings under different condition of germination, transplantation and cultivation. In season of 2019, the vegetation cycle of *Phytolacca americana* L. annual plants continued maximal 210 days (from March to November). The biometrical indexes of stems, racemes, fruits and seeds corresponded to similar characters reported by other scientists. The aqueous and hydroalcoholic extracts were obtained from ripe fresh fruits. The biggest amount of polyphenolic and colorant substances was found in aqueous extracts. The antioxidant activity of *Phytolacca americana* L. fruit extracts, evaluated *in vitro* by potentiometric procedure against the peroxyl free radicals, was in direct proportion to the content of polyphenols (r^2=0.9563) and colorant substances (r^2=0.9808). The colorant substances from American pokeweed fruits possessed the high antioxidant activity, IC_{50} was equal to 259.65±2.60mg/l.

Keywords: *American pokeweed, growth particularities, fruit, colorant substances.*

INTRODUCTION

American pokeweed (*Phytolacca americana* L.) is the herbaceous perennial plant with multipurpose utilisation. Due to annual renewal of the aerial part (plants resprout from a large fleshy taproot), rapid growth ability, and attractive grape-like fruits the American pokeweed is wide used as an ornamental plant in horticulture and botanical gardens. American pokeweed berries are reported to be a good source of food for songbirds and other bird species and small animals. Young leaves and stems of American pokeweed in spring are harvested in order to prepare the different food of Native-American, African-American and Southern cuisines (Balogh and Juhasz, 2008). The extracts from aerial part and roots of *Phytolacca americana* L. exhibit various pharmacological effects (anti-inflammatory, antifungal, antiproliferative activity), which allowed the development and standardization some homeopathic preparations. The matrix tinctures derived from the fresh roots and recommended for treatment of mumps, arthritis and various skin diseases were included in the Homeopathic Pharmacopeia of Germany and Brasilia. The Chinese Pharmacopeia describes the powder from dried roots as an allopathic medicine for carbuncles treatment, as well as a purgative, salve, bronchodilator and diuretic. The pokeweed antiviral protein (PAP) produced in leaves and seeds of *Phytolacca americana* L. possesses an increased antiviral and antifungal activities (Tumer *et al.,* 1999; Patra *et al*., 2014; Domashevskiy and Goss, 2015). Purified PAP was found to be a potent inhibitor of eukaryotic protein synthesis and viruses of several plant and animal. The mechanism of its cytotoxicity, PAP-induced disease resistance in plants and application to agriculture were described in review (Di and Tuner, 2015). Fruits and natural dyes accumulated in *Phytolacca americana* L. fruits have been used since the 17th century as an ingredient for pickled vegetable and red wines to give the products astringency, spice taste and attractive colour (Balogh and Juhasz, 2008; Florea and Donea, 2010). Actually, the possibilities of application the natural dyes of American pokeweed for food and textile industries are intensive studied (Liu *et al.*, 2014: Mchedlishvili *et al.*, 2014; Park and Jung, 2014; Belhadj, 2017). Thus, the all organ of American pokeweed plants contains the biologically active substances, but this species of plant and some useful chemical constituents, in our opinion, are not utilised sufficient effectively. In the Republic of Moldova and Slovak Republic, the plants of American pokeweed are propagated only in botanical collections. These plants could be reproduced by seeds, sprouts from a large fleshy taproot and micropropagation using various organ cuttings of plant (Florea and Donea, 2010; El-Minisy *et al.*, 2017). The purpose of this work was to study the growth particularities of American pokeweed plants propagated by seeds, and to establish the content and antioxidant activity of main constituents (polyphenols and colorant substances) in ripe fruits.

MATERIALS AND METHODS

Seeds of *Phytolacca americana* L. were collected from plants growing on the territory of the Republic of Moldova (Chisinau) and the Slovak Republic (Nitra). A part of seeds was harvested in September in the end of seasons from fresh ripe fruits, and other part of seeds - in February from fruits dried and overwintered on the shrubs. The seeds were stored in the same conditions: at room temperature 18-20 ºC, in darkness. The morpho-biological features of *Phytolacca americana* L. plants were studied in the season of 2019. In the last decade of March 2019, the seeds were sown for germination under different conditions: directly in the field, and on pallets in the greenhouse to obtain the seedlings. Planting density in the field was 80 cm between rows and 50 cm between plants. Field experiments were carried out at the research station of Institute of Genetics and Plant Protection in Chisinau area of Republic of Moldova (lat. 47^{o}01', long. 28^{o}75', alt. 85 m above sea level). The aqueous, ethyl alcoholic (96%) and hydroalcoholic (40%) extracts were obtained from ripe fresh fruits. The total polyphenolic content in extracts was appreciated by Folin-Ciocalteu method in equivalent to gallic acid (Singleton *et al.*, 1999). The concentration of colorant substances was determined using UV-Vis spectrophotometry at 520 nm. Radical scavenging activity of fruit extracts was detected *in vitro* by potentiometric procedure (Sano *et al*., 2003) in our modification (Ivanova, 2016), using 2,2'-azobis (2-amidinopropane) dihydrochloride as generator of reactive peroxyl ROO· radicals. Antioxidant activity were calculated from dose-activity curves and expressed as IC_{50} (concentration that inhibit 50% of free radicals) index of extracts.

RESULTS AND DISCUSSION

The first seedlings growing in the field conditions were observed after 55 days from sowing date. No differences in rate of germination between fresh collected and overwintered seeds was determined. It is necessary to note that in greenhouse conditions the seedlings appeared earlier than in the field for 10-15 days. Seedlings were represented by a small orthotropic shoot 2-3 cm long with 3-4 leaves and weakly expressed internodes. In the last decade of May, the seedlings from greenhouse were transferred in the field. Despite the fact that the various recommendations for cultivation of *P. americana* mentioned that the seedlings do not like transplantation, in our experiments transferred seedlings easily adapted to the new growing conditions. During 25-30 days after transferring, the young generative plants had one leafy orthotropic stem with length of 40-50 cm and 2-4 side branches (fig.1 b). The stems are straight, thick, juicy, green at beginning of growth and reddish as they grow older. The leaves were of different sizes (from 10 to 30 cm), which varied depending on the leaf position on the stem. The pokeweed leaves are green, oblong or ovate-lanceolate in shape, at the apex acute (fig.1 c). Damaged leaves exude a specific aroma.

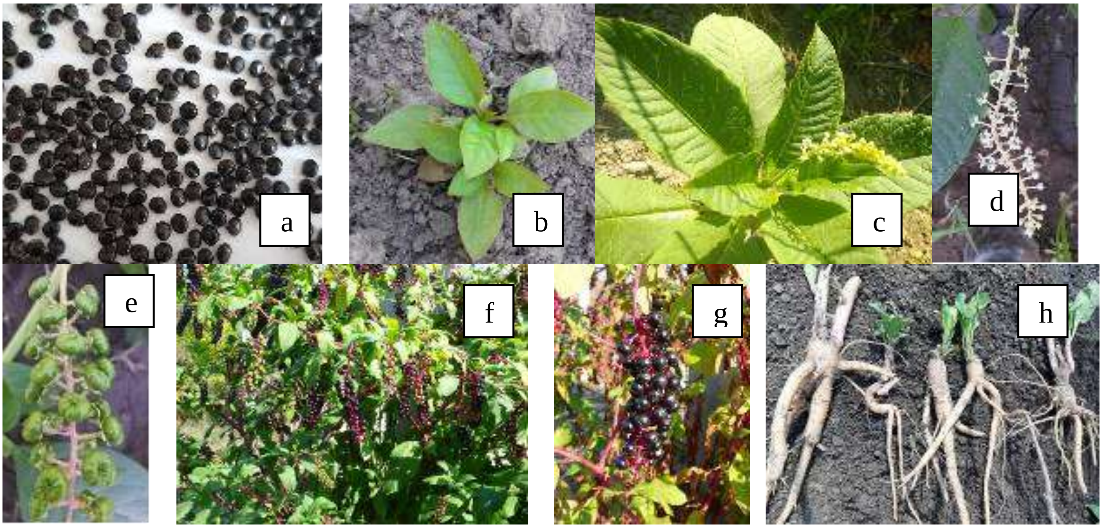

Figure 1. *Phytolacca americana* L.: a) seeds; b) seedlings; c) flowering plant; d) flower; e) raceme with green berries; f) plant in fruits ripening stage; g) ripe fruit; h) roots.

At the mid-June, the flowering stage began (fig.1 c), which lasted until the first decade of September. American pokeweed inflorescences are racemose, multi-flowered, more frequent 10-15cm long. The flowers are white, small (diameter 0.5 cm, length 2.5 cm), bisexual (fig.1d). Flower racemes was typically erect when in bloom but begin to droop as the fruit develops. A distinctive feature of *Phytolacca americana* L. from other species of *Phytolacca* is that the inflorescences are inclined under the heaviness of initially light green and then reddish-purple berries (fig.1e, f, g). Thus, already year-old American pokeweed plants bloomed in abundance and bore fruit. In our experiments the plants did not get sick and did not suffer from pests. Thus, in vegetation season of 2019 the annual plants of *Phytolacca americana* L. demonstrated the following duration of growth stages: 40-55 days for seeds germination, 25-35 days of active growth of stem, branch, and leaf areas, 55-60 days of flowering and 50-60 days of fruits ripening. Flowers and fruits were presented simultaneously at different stages of development. In general, the vegetation cycle persisted from 160 to 210 days, or from March to November. In pedoclimatic conditions of the Republic of Moldova, the seedlings developed in the soil a multi-headed short rhizome with a thick, fleshy and fusiform rod roots, from which the plants will resprout in spring of next years (fig.1 h).

Previous studies on the propagation of *Phytolacca americana* L. in the Republic of Moldova (Florea and Donea, 2010) led to the conclusion that there is a direct correlation between biometric characteristics, such as the height and mass of the generative stem, the number of leaves, inflorescences and fruits on it. The strongest correlation was revealed between the height of the generative stems and other studied characteristics of productivity. One generative stem of 1.2-2.6m tall had from 16 to 39 number of inflorescences and produced 696-2083 of berries (Florea and Donea, 2010). Each berry contains about nine seeds; consequently, one plant of American pokeweed can produce 3,500-18,000 seeds annually. In order to develop these investigations, the length of American pokeweed racemes, weight of berries from one raceme, seeds size and 1000 seeds weight were determined. In 2019 season the length of racemes varied from 8.8cm to 17.5cm, 76.2% of racemes had the length in limits of 15.00±1.85cm. The diameter of American pokeweed berries was 8.73±0.25mm. Weight of berries from one raceme in average was 40.68±3.27g. A positive correlation was found between the length of raceme and the weight of berries per raceme. American pokeweed seeds are black, reniform-orbicular, shining, smooth (fig.1a). The size of harvested seeds in season of 2019 was 2.78±0.09cm of length and 1.80±0.20cm of width. Seeds weight was 6.45±0.02g/1000 seeds. Therefore, determined morpho-biological features of *Phytolacca americana* L. plants reproduced from seeds and grown in season of 2019 under the pedoclimatic conditions of the Republic of Moldova did not differ from similar data reported by other scientists (Balogh and Juhasz, 2008; Dzadzieva, 2011). The antioxidant activity of extracts from *Phytolacca americana* L. fruits is depending on extraction procedures and content of polyphenolics (Brindza *et al.*, 2014; Ivanova, 2019). The alcoholic and hydroalcoholic extracts from American pokeweed fruits contained 5.80±0.62mg and 8.33±0.42mg of total polyphenols per g of dry residue, correspondingly. The biggest amount of polyphenolic substances was extracted by water, in aqueous extracts their content was equal 17.63±0.61mg/g. However, it was reported (Nabavi *et al.*, 2009; Zheleva-Dimitrova, 2013) that in extracts from pokeweed fruits obtained by percolation using methanol the content of polyphenolic substances could reached to 102.11±6.37mg equivalent to gallic acid or 174.76±0.74 mg pyrogallol equivalent per g dry extract.

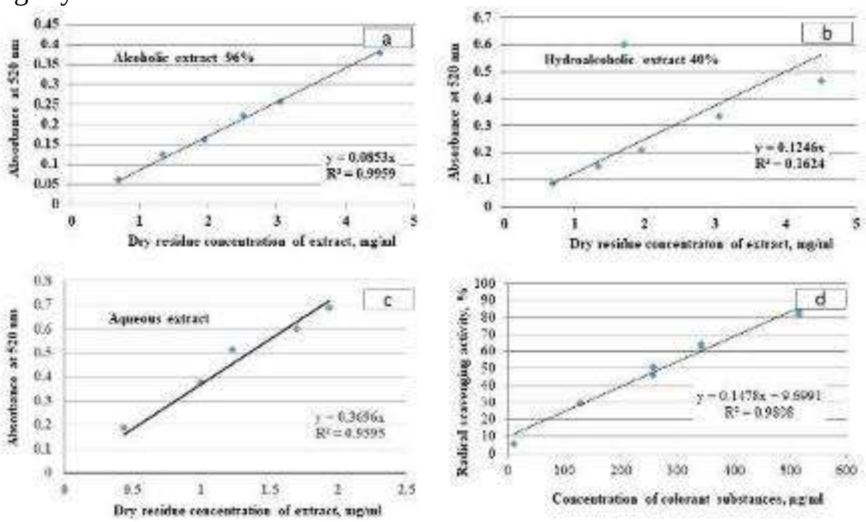

Figure 2. Dependence of absorbance on dry residue concentration of alcoholic (a), hydroalcoholic (b) and aqueous (c) extracts and dose-activity curve of colorant substances (d).

Extraction procedures have also had a major impact on content of colorant substances in fruits extracts (fig. 2 a, b, c). The dependences of extracts absorbance regarding to colorant substances on concentration of dry residue in limits from zero to 4.5 mg/ml were linear with a good approximation (0.9595-0.9959). The absorbance values of different extracts were compared regarding to the same concentration of dry residue, as follows: the absorbance of aqueous extract with concentration 2mg/ml of dry residue was equal 0.7; hydroalcoholic and alcoholic extracts – 0.2 and 0.16, respectively. Thus, aqueous extracts contained 3.5-4.3 times more colorant substances than hydroalcoholic and alcoholic extracts.

The relationship between content of colorant substances and antioxidant activity of fruits extracts was determined (fig.2 d). The colorant substances from American pokeweed fruits exhibited the high radical scavenging activity against peroxyl radicals; IC_{50} was equal to 259.65±2.60μg/ml. It should be noted that the reported data on radical scavenging activity of integrated extracts from American pokeweed fruits against DPPH free radicals differ considerable from IC_{50}=62.0±2.1 μg/ml (Nabavi *et al.*, 2009) to IC_{50}=412.06μg/ml (Zheleva-Dimitrova, 2013). I was determined that the antioxidant activity of natural colorant from American pokeweed fruits was stable during 12 months of storage (Mchedlishvili *et al.*, 2014). In our research the antioxidant activity of fruits extract was in direct dependences on content of both polyphenols (R^2=0.9563) and colorant substances (R^2=0.9808). Thus, the American pokeweed fruits contain the chemical constituents such as polyphenolic and colorant compounds, which directly influence on their biological activity.

CONCLUSION

The growth particularities of *Phytolacca americana* L. plants in pedoclimatic conditions of the Republic of Moldova were studied. American pokeweed demonstrated the excellent adaptivity of seeds and seedlings under different condition of germination, transplantation and cultivation. Taking into account the abundant growth of these perennial plants by seasonal renewal and the accumulation of valuable chemical constituents with biological activities, we could recommend to initiate the introduction of *Phytolacca americana* L. into the culture. The polyphenolic and colorant substances from American pokeweed fruits exhibit the high radical scavenging activity and can be used as ingredients in food technologies.

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Original Scientific paper

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GLOBAL LARGE-SCALE LAND INVESTMENT IN AFRICA: IMPLICATIONS FOR THE ENVIRONMENT

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ABSTRACT

The attainment of sustainable development goals (SDGs) in Africa will depend in part on its endowment, productivity and management of the land resource. Thus, due to the multipurpose usage of the land, there is more interest in its acquisition and usage, which often lead to competition among investors. More so, the intensive use of land for economic activities often impacts on the environment. This has implication for the target countries' sustainable development. It is on this basis that this study investigates the effects of large-scale land investments on the environment. The study adopts the sample selection model to find that at the decision to invest, there is the tendency the environment gets more deplorable while the foreign investors sustainably use the land and this is not the case for domestic investors. At the actual large-scale land investment level, the foreign large-scale land investment has adverse effects on the environment, but they maintain sustainable use of land, while the domestic large-scale investment negatively impacts on both the environment and the sustainable land use. Climate change impeded the availability of large-scale land. Thus, although the large-scale land investments could mitigate the challenges of national food insecurity, there should be intense efforts by the government to continuously monitor and regulate the activities of these investors to conform with global environmental best practices.

Keywords: *Environment, Land Investments, Large-scale, Gravity model, Africa.*

INTRODUCTION

The economic potentials of countries in part depend on their natural resources' endowment and productive utilization. Land ownership or otherwise indicates the status of an economic agent in society. More so, the attainment of SDGs by developing countries, particularly Africa will depend in part on their endowment, productivity and management of the land resource. More than half of SDGs target goals are directly related to this natural resource. Hence, the importance of land to the sustainable development aspirations of countries, particularly resource endowed, cannot be overemphasized. Land remains an invaluable natural resource that is precious to man, but it is non-renewable. The non-renewability of land and the scarcity of fertile land led to its increasing demand. More so, the multipurpose usage of the land enhances interest in its acquisition. Moreover, owing to the effects of mineral exploration, urbanization, environmental degradation, etc, the availability of fertile and arable land becomes increasingly difficult. This has implication for sustainable development, especially for the vulnerable people in the rural areas, since they depend largely on land for their livelihood. Many people in Africa depend on land for their economic activities and/or livelihood. This is because it is from it that food is provided, shelters are constructed, infrastructures are laid and other valuable minerals are found. Kareem (2014) finds that 52% of the total employment in Africa is in the agricultural sector. Thus, access to land has become more competitive among large-scale land investors in Africa, while the availability of fertile and productive land is becoming increasingly difficult owing to the influx of large-scale land investors to Africa.

Many of the plantation investments caused environmental degradation without tangible rural development. This led to limited access to fertile land which necessitated frequent struggle for the acquisition of arable land and conflicts over the best usage. Moreover, the large-scale land investments could lead to acrimony and crisis between the investors and landowners, communities and smallholder farmers. There are great possibilities that these acquisitions could crowd-out subsistence farmers that often make use of fallow land. To prevent these problems, the government regulates and manages land acquisitions to ensure sustainable use of the precious resource. Besides, government institutions are strengthened to monitor and evaluate these acquisitions to ensure the best environmental practices and standards across the board. Available evidence indicates that there are a lot of challenges to land governance, while the preponderance of controversies, public outcry, crowding-out and welfare depletion due to the land investments is worrisome.

Furthermore, the recent economic events, particularly the commodity crisis of 2007-2008, have shown that there had been increasing demand for land in the global south, especially in Africa, which affected the availability of fertile land. Evidence has shown that the demand for land has increased over time and the trend is expected to continue in the future, especially for Africa that has about 5% of its total agricultural areas invested, which is like the territory of Kenya (Kareem, 2018). Although some African countries promote agricultural investment, the Comprehensive Africa Agricultural Development Programme of the African Union Commission specifically enjoined national agricultural investment as part of its programme – at least 10% of the national budget (Kareem, 2016a). This cannot be the main reason for the volume of land investments. Other factors could have accounted for the investments exogenously, in which external agents such as the foreign investors are deeply involved, especially during the spike in global commodity prices – foreignization of space (Zoomers, 2010).

Studies in this area of research often focus on the effects of land deals, acquisition, transaction, ownership, tenure and reform on both micro and macroeconomic variables without determining the sustainability of the land investments and its environmental impact (Deininger et al., 2015; Deininger and Byerlee, 2012). A segment of the literature examines the effects of ownership of land and land grab on development in developing countries and normatively reflect on the drivers of the land investment. Similarly, in the context of Africa, some studies evaluate agricultural investments and international land deals in Africa to determine whether the investment is a land grab or development opportunity (Schoneveld, 2014; Kareem, 2016b). Some studies have econometrically determines the impact of foreign land deals in Africa on agricultural trade (Kareem, 2018; Arezki et al., 2015). Thus, a critical review of the literature indicates that only scanty empirical studies exist on the effects of large-scale land investments on the environment. Majority of the related literature either apply normative, qualitative or descriptive analysis (Di Matteo and Schoneveld, 2016).

It is on this basis that this study investigates the extent to which large-scale land investments impacts on the environment in Africa using an augmented Helpman, Melitz and Rubenstein model. This study uses data from the Land Matrix for the large-scale land investments and got other data from the World Development Indicators of the World Bank. This model is a selection bias model with firms' heterogeneity which uses a Poisson.

MATERIALS AND METHODS

The data for the empirical analysis in this study is sourced from the Land Matrix, which is used in the background and more specifically the model's land investment contract size. These data contain 702 land investment deals that cut across the period of 2000 to 2015. Other sources of data are the World Development Indicators of the World Bank, World Integrated Trade Solution database of the World Bank, and time and date website for bilateral distance.

The study's methodological framework is derived from Helpman, Malitz and Rubinstein (2008) – hereafter called HMR - selection model that includes firm heterogeneity and correct for sample selection bias and specification error with nonrandom zero[1]. This study departs from previous studies by adopting the HMR model to the bilateral investments' framework. Large-scale land investments are carried out with different outcomes; there are land transactions that are concluded, failed deals, some under negotiations, and there are expressions of interest. In all the transaction outcomes, only those that have been concluded are the actual and positive investments, however, others have no value of the outcome and thereby at present no investment, but in the future, the transaction might be concluded especially for those under negotiation. Consideration of only the concluded transactions (positive investment) will lead to selection bias. Thus, the HMR is adopted to control for both the sample selection bias and the investors' countries heterogeneity bias with adequate consideration for bilateral zero investment flows

[1] See Kareem and Kareem (2014) and Helpman, et al. (2008) for a comprehensive description of the model

in a two-step estimation procedure. First-step estimates a binary equation (probit regression) for the probability of large-scale land investment at the heterogeneous firm/country level, which is the extensive margin of investment – the decision to invest. The second step involves a count model of investment estimated in its logarithm form and entails using the predicted probabilities obtained in the first step to estimate the effects on large-scale land investments' sustainable environmental land use (intensive margin of investment). The model is specified as follows:

$$T_{ijt} = \beta_1 + \gamma_{it} + \rho_{jt} + C_{ij}\vartheta + \pi E_{ijt} + \varepsilon_{ijt} \qquad (1)$$

where T_{ijt} is a binary variable that equals 1 if the number of land deals from country i to j at time t is nonzero; otherwise, it is 0. The intercept is β_1; the investor and target countries fixed effects are γ_{it} and ρ_{jt}, respectively; C_{ij} is a vector of pair-varying control variables such as distance, language, arable land, institutions and governance variables as well as others included. E_{ijt} is the exclusion variable[2] that does not enter the second – stage regression.

The second-stage equation relies on a standard count model represented in a general form of a conditional probability function as:

$$\Pr(Y_{ijpt} = y_{ijt} \mid x_i) = \frac{\exp(-\exp(x'_{ijt}ß))\exp(y_{itj}x'_{ijt}ß)}{y_{ijt}!} \qquad (2)$$

where subscripts i, j, p and t denote investor, target country, intention/sector and time respectively; y is the count variable, in this case, the available fertile land owing to the environmental large-scale land degradation in Africa; x is the vector of independent variables of the model and β is the vector of the associated parameters. The model is specified as:

$$Fertile\_land_{ijpk} = \beta_0 + \beta_1 Land\_deals_{ij} + \beta_1 Demographic_j + \beta_2 Economic\_size_{ij} + \beta_3 Trade_j + \beta_4 Pr\,o\,duction_j + \beta_5 Institution_j + \beta_6 Governance_j + \beta_7 Natural\_resources_{jt} + \beta_8 Energy_j + \beta_8 Security\_safety_j + \pi_{ij} + \delta_i + \delta_j + \delta_s + \mu_{ijpt}) \qquad (3)$$

From equation 3, the dependent variable is the available fertile land, a measure of the environment, owing to the degradation of the environment from the activities of the large-scale land investors. The parameters δ_i, δ_j and δ_s are the investor country, target country and sector/intention of investment fixed effects. The investor and target countries fixed effects stand for the multilateral investment resistance variables. Finally, π_{ij} is the inverse Mill ratio that is derived from the first-step regression, which is used in the second step. The inverse Mill ratio is the ratio of the probability density function (PDF) and the cumulative density function (CDF) of the normal distribution, which is evaluated at the predicted outcomes

[2] For further reading on exclusion variable see Kareem (2016a)

divided by the standard error of the probit estimation. A Poisson estimator is employed based on the fact that the assumption of equi-dispersion of the Poisson estimator is unlikely to hold.
The Land Matrix provided the data used in the background and more specifically the model's land investment contract size. These data contain 702 land investment deals that cut across the period of 2000 to 2015. Other sources of data are the World Development Indicators of the World Bank, World Integrated Trade Solution database of the World Bank, and time and date website for bilateral distance.

EMPIRICAL RESULTS

Extensive Margin of Large-Scale Land Investment

The second column of table 1 shows that the decision to conclude more land deals would possibly increase the rate by which the environment is degraded. The environment would be depleted by 0.32% for every per cent decision made to increase land deals. Hence, the environment could be degraded as the probability and/or the decision to conclude more land deals increases. The estimates indicate that the probability of the large-scale land investors to intensively apply fertilizer on the land would have significant adverse effects on the environment such that a unit increase in fertilizer application adversely affects the environment by 0.6%. The intensity of fertilizer application, especially the chemical fertilizers, tends to hardened the soil and thereby strengthened pesticides as well as pollute water and air and thus, release greenhouse gases that are hazardous to human health and the environment. The energy intensity would significantly and negatively impact on the environment at this margin of land investment such that a per cent increase in the energy intensity would probably make the environment deplorable by 1.5% for pool estimates while 0.6% and 0.4% are the foreign and domestic land investments, respectively. The volume of farm yields which shows the land fertility often propel large-scale investors to such destinations, which in turn have effects on the soil's nutrients and greenhouse gas emission. The estimate indicates the farm yields, measured by cereal yields, does not adversely impact on the environment (-1.5) The implies that the tendency to apply modern technologies on the farm would have adverse effects on the soil composition and the environment. Precipitation, a measure of the climatic condition, which ought to increase the fertility of the land have a significant adverse effect on the environment for both the pool and foreign estimates while the domestic estimate is insignificant. Institutions, measures by the business regulatory environment, tend to contribute to the deplorable state of the environment in Africa. Since, the land regulatory agencies and governance in Africa are weak (see Kareem, 2018), this would lead to an inadequate contract and standard enforcement and thereby propelling large-scale land investments that utilize the land in such a way that would affect the environment and land sustainability – against global best practices.

Table 1. Extensive and intensive margins

Variable	Extensive margin	Intensive margin
Land deals	0.3247[a] (0.0621)	0.3548[a] (0.0579)
Fertilizer	0.5885[a] (0.1510)	-0.3993[a] (0.0047)
Energy intensity	1.5137[b] (0.6577)	0.3501[a] (0.0115)
Yield cereals	-1.4588[a] (0.2618)	0.1190[a] (0.0275)
Precipitation	1.9069[a] (0.2598)	-0.5994[a] (0.0308)
Business regulatory environment	2.1955[a] (0.5793)	0.0150 (0.0235)
GDP target country	0.2222[a] (0.0663)	0.2406[a] (0.071)
Language	-0.0946[a] (0.0250)	
Inverse Mill Ratio		-0.6551[a] (0.0222)
Constant	-7.2987[a] (2.3565)	-0.0772[a] (0.0812)
Observation	18,244	18,474
Wald Chi2	2183.87 (0.0000)	
Pseudo R^2		0.6112

Source: Computed. Note that a, b and c stand for 1, 5 and 10% significant levels. The figures in parentheses are the robust standard errors. All variables are in log form except the dummy variables. The fixed effects are included.

Intensive Margin of the Large-Scale Land Investment

In terms of the actual large-scale land investments, the rise in the land deals leads to a significant depletion in the environment to the extent that a per cent rise in the land transactions decreases land fertility by 0.35% owing to the economic activities on the land. The intensiveness of the use of fertilizers significantly did not adversely affect the environment, which implies that chemical fertilizers and other environmental damaging chemical were not applied to the land. Hence, the fertilizers usage tends to nourish the soil such that a per cent increase in fertilizers application by all the investors improve the soil nutrient by 0.4% and the magnitude of the impact is same for all categories of land investors. However, the energy intensity significantly leads to environmental degradation with the largest impact magnitude from the domestic land investors (0.6) compared to 0.3 for other investors. The farm yields, measured by the cereal yields, significantly lead to the deplorable environment because as more yields are harvested there is a tendency to further cultivate the land and other exploration which might reduce the land nutrients and fertility and thereby make the environment deplorable. The magnitude of the deplorability of the environment due to farm yield is more pronounced in the domestic land investment (0.6) than the foreign which is 0.2. Furthermore, the climatic condition, measured by precipitation, significantly did not make the large-scale land investment at this margin to be environment degradable. The more the precipitation the higher environment sustainability by all categories of large-scale land investors. The institutions are significant and tend to protect the environment for sustainable land utilization. The business regulatory environment indicates that despite the inadequate institutional capacity in Africa, contract enforcement and land governance is such that sustainable land use and environmental protection is ensured.

THE SUMMARY AND CONCLUSION

The paucity of literature that has empirically investigate the impact of large-scale land investments on environmental sustainability motivates this study. The results suggest that the activities of large-scale land investors impacted adversely on the environment. The study further finds that at the decision to invest (extensive margin), there is the tendency that the environment gets more deplorable. At the actual large-scale land investment level (intensive margin), the foreign large-scale land investments have adverse effects on the environment but they maintain sustainable use of the land, while the domestic large-scale investment negatively impacted on both the environment and the sustainable land use. Climate change impeded the availability of large-scale land, especially for agri-food production and other land uses such as forestry, conservation, renewable energy and tourism. Thus, this study concludes that large-scale land deals as being obtained in Africa are not environmental friendly. The investors need to adopt international best practices and standards in the implementation of their economic activities to use the land sustainably and not degrade the land.

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Original Scientific paper

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THE EFFECT OF DIFFERENT NEW BEDDING MATERIALS ON AMMONIA EMISSION FROM DAIRY COW SLURRY

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ABSTRACT

Agriculture is the most significant source of Ammonia emission that causes e.g. loss of Nitrogen from agricultural systems. Manure is the main source of Ammonia emissions and causes losses in the nutrient cycles of agriculture as well as local odour nuisance. By using different bedding materials, it is possible to reduce both the Ammonia emissions and to improve the cycling of nutrient. Peat is known as an effective litter material but its use as a virtually non-renewable resource is questionable. Therefore, we need to find new bedding materials to replace peat. In this study, the effect of ten different industrial by-products, reeds and stalks to reduce Ammonia emissions was tested in laboratory in January 2020. Dairy cow slurry and bedding materials were mixed in a volume ratio of 4:1. The Ammonia emission was measured for two weeks once or twice a day. Measurements were performed with a photoacoustic method. The results show that all tested materials reduce the Ammonia emission from the cow slurry used. Interesting new materials to substitute peat are zero fiber and briquetted textile waste. Wheat bran, pellets made of reed canary grass and chopped bulrush had the best effect which is at the same level as that of peat. However, no statistically significant differences between the calculated emission rates were found.

Keywords: *Bedding material, Peat, Ammonia emission, Animal production, Cow slurry.*

INTRODUCTION

Agriculture is the most significant source of Ammonia emission that causes e.g. loss of Nitrogen from agricultural systems. The emission of Ammonia from animal manures is the largest source of atmospheric Ammonia in Europe (Buijsman et al., 1987). Ammonia volatilization from animal husbandry contributes to the total nitrogen deposition, causing acidification and eutrophication. Ammonia emissions cause also losses in the nutrient cycles of agriculture as well as local odour nuisance.

The animal protection law in Finland, legislated in 1996, prescribes that bedding materials have to be used to a greater extend in animal production than previously. Bedding material is essential from the point of view of animal health and welfare. This substrate can be an organic material like wood or plant-based material like straw or inorganic material like clay and sand and should generally be a good absorbent, easily available, comfortable and nontoxic to animals. By using different bedding materials, it is possible to reduce both the Ammonia emissions and to improve the cycling of nutrients.

In Finland, the main bedding materials in use are straw, peat and wood shavings (Alasuutari et al., 2015). Peat is known as an effective litter material to reduce Ammonia emission (Hellstedt et al., 2017) but its use as a virtually non-renewable resource is questionable. Therefore, we need to find new materials with good Ammonia binding capacity to be used as bedding and to replace peat.

MATERIAL AND METHODS

The effect of different bedding materials on Ammonia emissions was tested on January 2020. The materials were selected based on their available in Finland. The test was made in a laboratory, where the average temperature was 18.6 ˚C (+/- 0.6 ˚C) and the relative humidity was 36 % (+/- 5.0 %), Figure 1.

The properties of the raw materials used in the tests are presented in Table 1. The analyses were made in the laboratory of Natural Resources institute Finland according to standardised methods. Dairy cow slurry and bedding materials were mixed in a volume ratio of 4:1. The ratio was the same as the one used by Airaksinen et al. (2001). Uncovered buckets of 30 litres were filled with 10 litres of slurry and 2.5 litres of bedding materials. The slurry and bedding materials were thoroughly mixed and left standing for one hour before starting the measurements. All the tests were performed in three replicates.

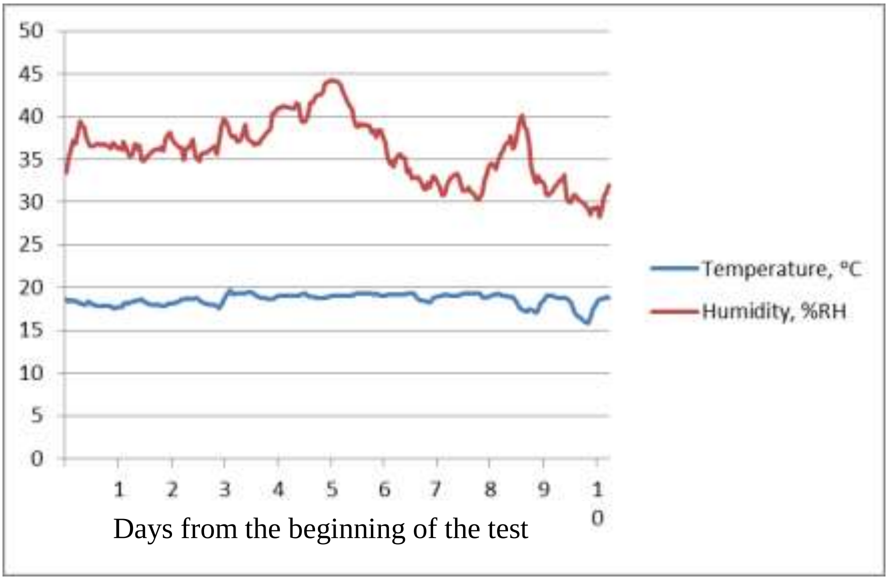

Figure 1. Average temperature and relative humidity during the laboratory test

Table 1. The properties of the materials used in the laboratory test

	DM	Ammonium-N	Total-N, Kjeldahl	P	pH (the aqueous extract)
	%	g/kg DM	g/kg DM	g/kg DM	
Rapeseed straw	88.84	0.12	8.25	1.48	6.61
Fiber hemp	90.00	0.01	4.09	0.62	5.46
Reed canary grass, pellets	90.25	0.07	7.61	1.40	5.48
Reed canary grass, chopped	82.86	0.53	10.37	1.38	5.65
Common reed, chopped	84.65	0.25	6.9	0.62	5.05
Pelletized sawdust, "Murukuivike"	89.57	0.01	0.57	0.06	5.56
Briquetted textile waste	96.36	0.02	1.02	0.05	7.00
Zero fiber	42.76	0.00	0.42	0.14	9.09
Wheat bran	88.68	0.05	28.47	13.53	6.83
Peat	58.57	0.07	8.79	0.27	3.98
Bulrush	84.72	0.05	5.82	1.03	6.97
Cow slurry	6.19	1.06	2.55	8.48	8.44

The amount of Ammonia from the mixtures was measured using a photoacoustic gas analyser (Innova™ Multi-gas analyser). The measuring period was four minutes and there were three replicates of these four-minute periods per one measurement for each material. The results were calculated as mean of these three replicates. The Ammonia emission was measured once for the first day, twice a day for the following three days, and thereafter once a day for five days. From the results, the mean and standard deviation of the three replicates were calculated.The significance of differences was calculated based on 95 % confidence level.

RESULTS AND DISCUSSION

The results show that all materials tested absorbed Ammonia, and the emissions were lower than that of raw cow slurry. Measured Ammonia emission was larger during the first week and diminished gradually on the second week, Figure 2. This is consistent with Andersson (1996) who also found the emission rates to be lower towards the end of his tests.

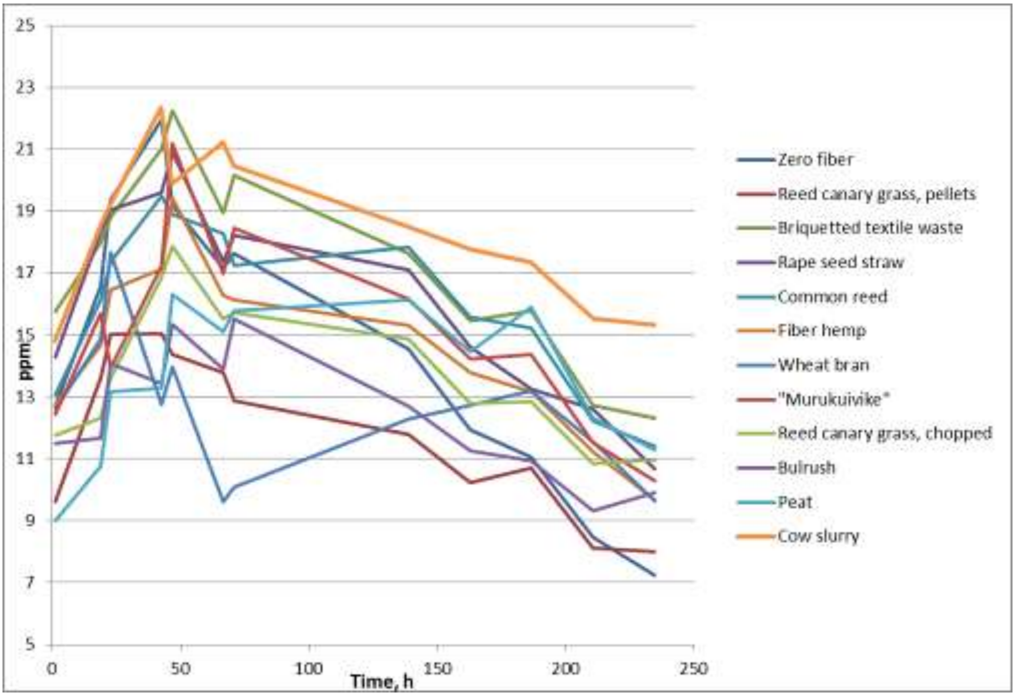

Figure 2. Measured Ammonia emission of the different bedding materials during the test period.

Calculation of the total Ammonia emission (NH_3 g/m^2) during the whole testing period shows that reed canary grass pellets, bulrush and wheat bran had the best diminishing effect on Ammonia emissions (Figure 30. All of these three had lower Ammonia emission than peat which is considered to be a good material to diminish Ammonia emissions due to its low pH. Materials consisting of chopped straw or stalk had larger Ammonia emissions compared to peat, which is consistent with the results of Jeppsson (1999). According to Andersson (1996) and Airaksinen et al. (2001) shredded newspaper had bigger Ammonia emission as compared to peat. Since zero fiber used in this research is a side product of paper production and there are also paper fibers included in it, our results are parallel. Similar to Airaksinen et al. (2001), we also found the emissions from fiber hemp and saw dust, which is the main component of Murukuivike^{TM}, to be larger than those of peat. However, in spite of the discovered differences in the calculated emission rates, none of the differences were statistically significant (Figure 4).

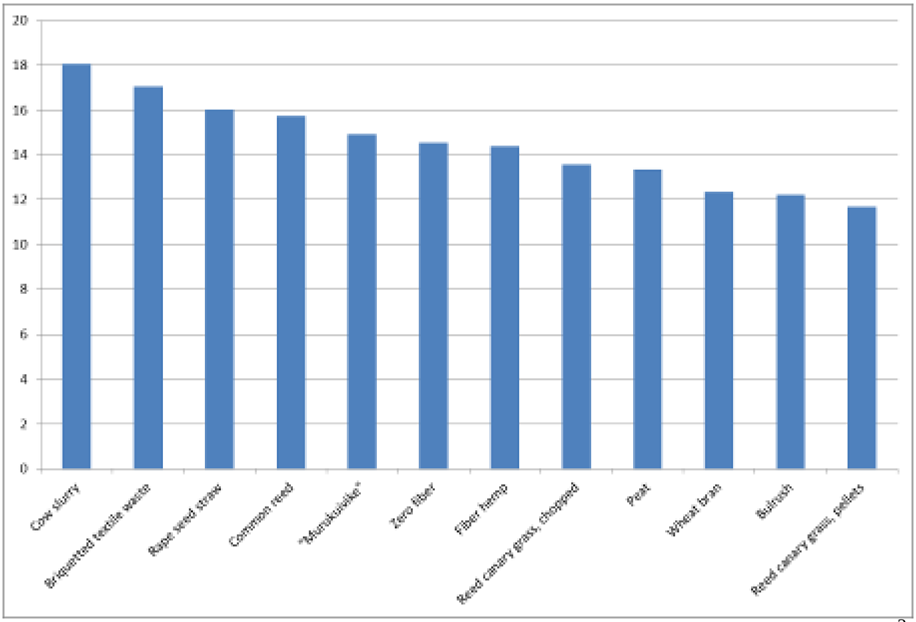

Figure 3. Calculated Ammonia emission during the whole test period, NH_3 g/m^2

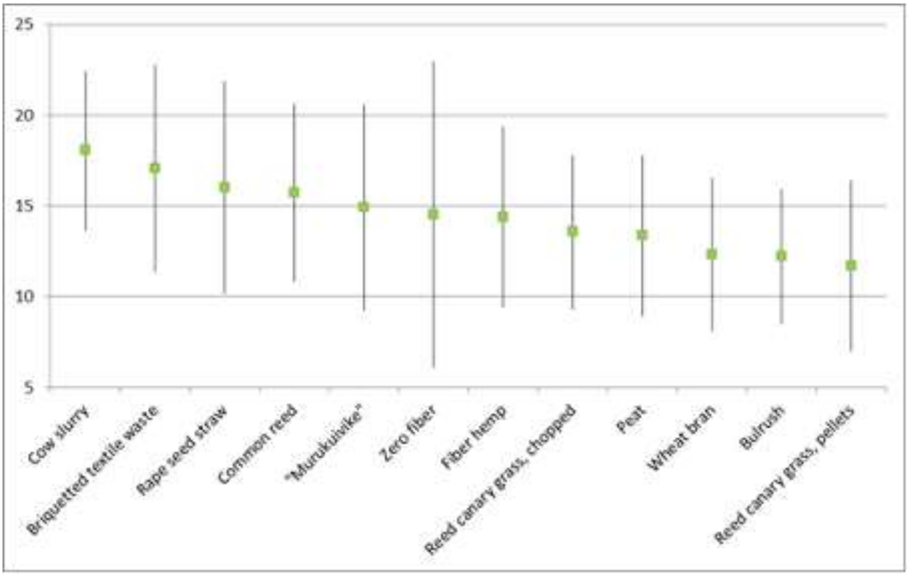

Figure 4. The calculated Ammonia emission results with 95% confidence level.

Changes in total mass and changes in ammonium-N and total-N contents of the slurry-bedding material mixtures during the test period are shown in Figure 5 and Figure 6. For all mixtures, the decrease in mass was less than 10 %. The decrement was highest for wheat bran, 9.6 %, and lowest for briquetted textile waste and bulrush, 6.7 %. For all the other materials the decrease in mass was between 6.8 % and 7.7 %.

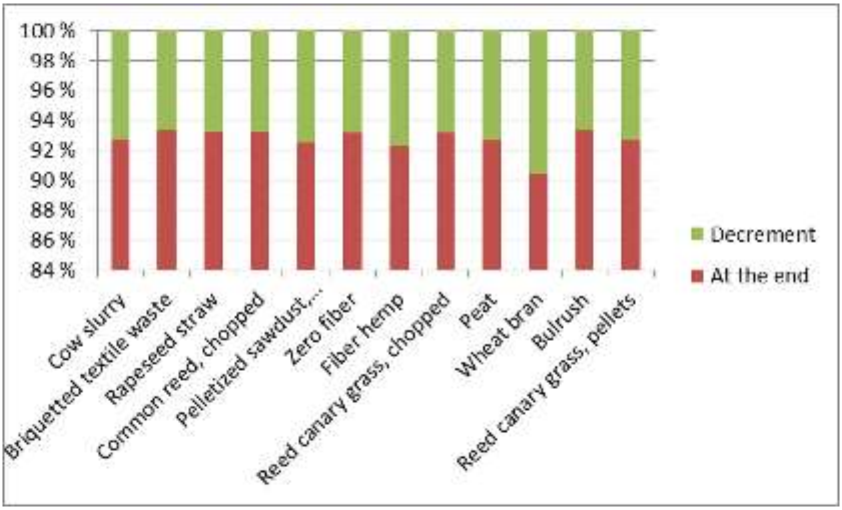

Figure 5. Changes in total mass in the buckets during the test period, as percentage of the initial value.

The decrease in Ammonium-N and total-N contents varied a lot between the materials. For pellets of reed canary grass and for briquettes of textile waste the loss of Ammonium-N was highest, 48 % and 30.6 % respectively. For the other materials the loss varied between 15.2 % and 23.1 %, except for wheat bran in which the amount of ammonium-N was increased by 7.8 % as compared with the value at the beginning of the test. The loss of total-N was highest for bulrush, 12.6 %, common reed, 11.8 %, pelletized sawdust, 11.6 %, zero fiber, 11.4 % and briquetted textile waste, 11.0 %. The loss was lowest for pellets of reed canary grass, 3.9 %, which is at the same level as that of raw cow slurry.

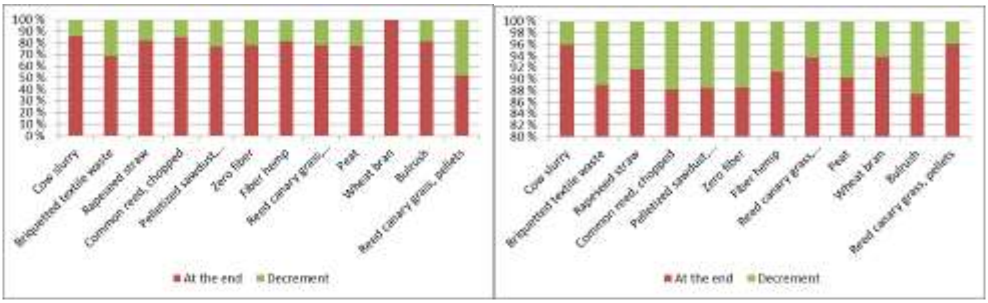

Figure 6. Changes in nutrient content of the slurry mixtures during the test period, as percentage of the initial value. Left Ammonium-N, right Total-N (Kjeldahl method).

CONCLUSIONS

According to the results, reed canary grass used as pellets or chopped fills the requirements for bedding material replacing peat. Also bulrush and fiber hemp seem to be very suitable for bedding material but their availability is not jet satisfactory. The wheat bran bound Ammonia well but it contains a lot of nutrients, both Nitrogen and Phosphorus. As such, its use as litter for manure is questionable because it would increase the nutrient content of the mixture.

Interesting new materials to substitute peat are zero fiber and briquetted textile waste. The availability of both these materials is good and they are also lacking applications. Before they can be recommended for use as bedding materials more research is needed on farm level.

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Original Scientific paper

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EFFECTS OF GREEN FERTILIZERS ON THE QUALITY STATUS AND PRODUCTION CAPACITY OF THE CAMBIC CHERNOZEM FROM MOLDOVA

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ABSTRACT

Chernozems Cambic from Central Moldova are subject to different forms of anthropogenic degradation. One of these is dehumification (loss of humus) and compaction of arable soil layers. In the recent situation regarding soil degradation of Moldova, the use of green fertilizers (leguminous) in crop rotation is the only possibility to remediate and maintain the quality status of the arable soils for the long term. Research was carried out over two farming years (2015-2016). In order to assess the quality status and production capacity of degraded cambic chernozems, two green mass harvest of vetch were incorporated into the soil as organic fertilizer on the field used for one year as an ”busy field” - sown with vetch two time (autumn 2014 and spring 2015), in the 5-field crop rotation (vetch-wheat-rapeseed-barley-sunflower). The research results showed that the incorporation into the soil by disking two harvests of green mass and vegetal debris of vetch (about 12,4 t ha^{-1}) as organic fertilizers led to the increase of humus content by 0.20%, compared to the control variant; to accumulation in the soil of 310 kg of nitrogen, of which 180 kg fixed from the atmosphere; synthesis of about 3 t ha^{-1} of humus or 1.7 t ha^{-1} of carbon; sequestration of about 6.3 t ha^{-1} of CO_2; a weakly positive balance of organic matter and nitrogen in the soil over 3-4 years was insured. On the plot where one harvest of green mass of vetch was incorporated into the soil as green fertilizers, the increase in the wheat harvest increased up 2.4 t ha^{-1}, and on the plot where two harvests of green mass of vetch were introduced into the soil, the harvest increase up 3.2 t ha^{-1}, the total harvest was 7.0 t ha^{-1}.

Key words: *Chernozem Cambic, Degradation, Organic matter, Green mass of vetch.*

INTRODUCTION

The contemporary arable cambic chernozems from Central Moldova inherited from the pedogenesis phase under the forest vegetation a differentiated textural profile, with high fine clay content in which the colloidal fraction dominates. Under the conditions of the existing agricultural system, these soils were subjected to the intensive process of dehumification and accelerated destruction of the arable layer. High clay content, dehumification and destruction accelerated secondary compaction of the arable layer (Canarache, 1990).The non-fertilization of chernozems with organic fertilizers, the insufficient use of chemical fertilizers, and the strong secondary compaction of the arable layer have led to a decrease in their production capacity. The main cause of the decreased compaction resistance of the arable layer of the investigated cambic chernozems is the insufficient flow of organic matter in soils. Organic fertilizers are not applied in these agricultural soils for the last three decades. The quantities of chemical and organicfertilizers used for crop fertilization are small and do not provide anequilibrated balance of nutrients in the soils (Cerbari, 2010).

Secondary production from agricultural crop harvesting, as a rule, is not incorporated into the soil and is used for other purposes or burned in fields. Under such conditions in all arable soils of Moldova, a profoundly negative balance of organic matter and nutrients in soil, especially phosphorus, was created. The unequilibrated balance of organic matter flow in the soil does not ensure large increases of agricultural crops yields due to the unfavourable physical state of the soils. A favorable long-term state of the soil physical quality can be created only by the existence of a permanent organic matter flow into the arable soil layer (Leah & Leah, 2018). The permanent organic matter flow can be ensured by systemically carrying out the following procedures in agricultural practice: the use of manure at a dose of 10-15 $t \cdot ha \cdot year^{-1}$ (currently only 50 $kg \cdot ha \cdot year^{-1}$ are used); repeated fallow (natural grassing or untilled) of degraded arable land (but this is impossible, that free land is not); the use of land under annual and perennial grasses (cannot be used extensively, since the livestock has been reduced by 6 times); The use of legumes (vetch, peas, beans, alfalfa, chickpeas, etc.) as an intermediate crop in field crop rotation - currently is the most rational procedure for degraded soils of Moldova (Leah, Cerbari, 2015).

The purpose of the researches was to test and demonstrate the phytotechnicalprocedure for increase the organic matter flow and remediate the degraded properties of chernozems in Central Moldova under the influence of green mass of vetch.

MATERIAL AND METHOD

The research object was chernozem cambic of Central Moldova from the agrochemical experimental station "Ivancea", Orhei district of the Institute of Pedology, Agrochemistry and Soil Protection "Nicolae Dimo". Variants of the experience: Witness (without the application of green fertilizers); with the incorporation of green fertilizers - vetch (*Vicia sativa*) – one harvest; with the incorporation into the soil – two harvests of vetch green mass. Predecessor culture was winter wheat. After the winter wheat harvest, in mid-July, the stubble was disking with the disc harrow at a depth of 8-12 cm (agrotechnical operation to perform the stubble field). The modification of the main properties of the arable soil layer was estimated by comparing the initial results obtained at the founding of experience (the control variant) with the results obtained on the experimental variants.

For modifications characteristic of the physico-chemical and agrochemical indicators of the arable cambic chernozem, classical methods adopted in the republic were used (granulometric composition (texture) - Pipette method, soil preparation by dispersion with Na pyrophosphate solution; bulk density – cylinder method; resistence to compactation – picnometr method; humus – Tiurin method; nitrogen – Kjieldali method; mobile phosphorus and potasium – Macighin method). The soil samples were collected from the depths: 0-10 cm, 10-20 cm, 20-35 cm, in which the soil properties were determined. To assess the quality status of investigated chernozem cambic, criteria developed by Canarache (1990) and Florea *et al.* (1987) were used.

The arable cambic chernozem is characterized by the profile type: *Ahp1→Ahp2→Ah→Bhw1→Bhw2→BCk1→BCk2→Ck* (Fig.1).

Fig. 1. Profile of the arable cambic chernozem

Fig.2. Structural fragment of the compacted horizon Ahp2 (20-30 cm)

Ahp1 (0-20 cm) – the arable layer, dark gray with a brown hue, dry, loamy-clayey, crumby-cloggy, very porous, weakly cracked, weakly compact, many roots and organic debris, clear passage to the next horizon.

Ahp2 (20-35 cm) – the postarable layer, black with brown hue, damp, loamy-clayey, compacted, prismatic-cloggy, prisms are practically without pores, cracked, thin and rare roots, clear passage to the next horizon (Fig. 2).

Ah (35-50 cm)– dark gray with brown hue, damp, loamy-clayey, nuts-form grains, small and medium aggregates, compacted, porous, small, medium and fine pores, thin roots, gradual passage to the next horizon.

Bhw1 (50-71 cm) – dark brown, loamy-clayey, nuts-form, the nuts break down into grains, compacted, small and fine pores, few roots, rarely - insect holes, gradual passage to the next horizon.

Bhw2 (71-95 cm) – light brown, damp, loamy-clayey, poorly structured, crumbles into nuts-form aggregates, compacted, small pores and fine, rare roots, gradual passage to the next horizon.

BCk1 (95-117 cm) – yellow with brown hue and white color micelles of carbonates, loamy-clayey, unstructured, breaks easily, the beginning of the illuvial carbonatic horizon, compacted, porous, medium and fine pores, some crotovines appear, gradually passing.

BCk2 (117-130 cm)–carbonatic alluvial horizon, light yellow-brown hue, micelles of carbonates, rare concretes, loamy-clayey, unstructured, lightly crushed, compacted, small and fine pores.

Ck (>130 cm) – yellow, with white pseudo micelles, less carbonate (micelle) neoformations than in the BC horizon, clayey-loamy, weakly compacted, porous, small and fine pores, many crotovines.

RESULTS AND DISCUSSIONS

The arable cambic chernozems are characterized by loamy-clayey texture and physical clay content in the humiferous horizons - 60% and in the horizons BC and C - 56-58%. The clay content in horizons A and B varies within the limits of 39-40%, and in the horizons BC and C - within the limits of 36-38% (Tab.1).

Table 1. Texture of the arable cambic chernozem, "Ivancea" Experimental Station

The horizon and the depth,cm	Fraction size, mm and content, %							
	1.0-0.25	1.0-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01	Ka*
Ahp 1 0-10	0.7	5.1	34.0	7.5	13.5	39.2	60.2	1.08
Ahp 1 10-20	0.7	6.9	32.2	7.6	13.6	39.0	60.2	1.08
Ahp2 20-35	0.8	6.8	32.0	7.8	13.4	39.2	60.4	1.08
Ah 35-50	0.5	6.7	32.8	7.1	13.2	39.7	60.0	1.10
Bhw1 50-71	0.4	7.4	32.1	8.4	11.9	39.8	60.1	1.10
Bhw2 71-95	0.6	5.6	33.6	8.3	12.4	39.5	60.2	1.09
BCw1 95-117	0.5	5.1	36.0	7.7	12.0	38.7	58.4	1.06
BCk2 117-130	0.5	5.0	38.1	7.4	12.4	36.6	56.4	1.01
Ck 130-150	0.5	5.1	38.3	7.5	12.5	36.1	56.1	1.00

Ka – the coefficient of argillisation (Cerbari, 2010)

The argillisation of the profile upper part of these soils is due to the modification of their hydrothermal regime in the process of use in arable. Texture as the main physical property of the soil plays an important role in determining most of the physical and chemical properties (Lal, 2011). The granulometric composition depend the soil production capacity, its agronomic and ameliorative characteristics, and the superior recovery technology. As texture is a virtually unchangeable property, agricultural and ameliorative technologies must adapt to the textural specificity of the soils (Berca, 2011).

The investigated cambic chernozems are characterized by high content of fine clay which, under conditions of dehumification and destruction of arable layer, is arranged for strong secondary compaction. According to Canarache (1990), *"loamy-clayey and clayey soils are characterized by large and very large quantities of inaccessible water, poor mechanical and thermal properties, tillage is hard, because have high swelling and contraction capacity, but have good chemical properties - high cation exchange and buffering capacity, high humus content"*. Other properties of these soils differ significantly depending on their structural state. The production capacity of the soils with fine texture, depending on the situations described above, varies, generally, from medium to small. These soils must be tilled in the optimum time, which is short, are generally receptive to deep ploughing; the necessary quality of the germinating bed is obtained with greater difficulty than on other soils (Cerbari, 2011). Thus, the investigated arable chernozems are a very difficult object of production in agriculture.

The total quantity of vetch green mass incorporated into the soil as green fertilizer (aerial and root mass) was 12.4 $t \cdot ha^{-1}$ of absolutely dry mass (Fig.3, Tab.2). The content of the main nutrients in the dry mass of vetch constituted: N - 2.5%, P_2O_5 -

0.6%, K_2O - 2.5%, C - 41.2% (Tab.2). During the vegetation period, the winter wheat plants on the experimental plots after the incorporation into the soil the vetch green mass differed from the wheat plants on the control plot by the following characteristics: more dark green color of leaves; greater thickness of the stem; the height of the spike with 20-30% higher than at the witness variant. The state of winter wheat plants at the beginning of July on the experimental plots, where the vetch green mass was incorporated in the soil is shown in Fig. 4.

According to the obtained data on the experimental variants, the change in the positive direction of the quality state of the structure is observed only for soil layer 0-10 (12) cm, formed by disking and mixing the artificial structural elements of this layer with the organic residues of the vetch. The modification of the quality status of the 0-10 cm arable layer under the incorporation in the soil of two green mass of vetch harvest is shown in Fig. 5.

Fig.3.The experimental strip of vetch - „busy field”

Fig.4. The winter wheat on the plots, where the vetch green mass was incorporated

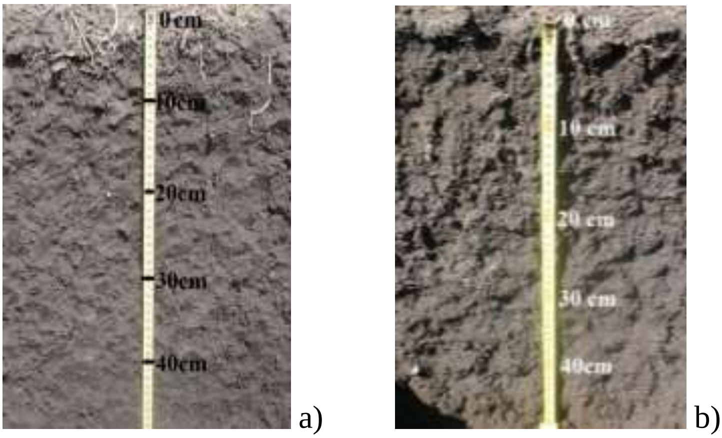

Fig.5. Chernozem cambic arable: *a) the control variant;*
b) the experimental variant, where two vetch harvests were incorporated into the soil

Table 2. Green mass harvest of vetch incorporated into the soil as fertilizer in 2015

Harvest	Green mass, $t \cdot ha^{-1}$	Humidity, % of green mass	Dry mass, $t \cdot ha^{-1}$	Ash	N	P_2O_5	K_2O	C
				% of dry mass				
Autumn vetch								
Main harvest	27.0	79.9	5.4	9.9	3.8	0.7	3.7	41.4
Vetch roots, total mass in 0-30 cm			2.2	14.8	1.8	0.5	1.5	41.1
Total aerial mass and roots of autumn vetch incorporated into the soil			7.6	11.3	3.2	0.6	3.1	41.3
Spring vetch								
Main harvest	9.6	64.2	3.4	10.3	1.5	0.5	1.5	40.9
Vetch roots, total mass in 0-30 cm			1.4	15.1	1.3	0.5	1.4	41.2
Total aerial mass and roots of spring vetch incorporated into the soil			4.8	11.7	1.4	0.5	1.5	411
Total vetch mass incorporated in the soil			***12.4***	***11.5***	***2.5***	***0.6***	***2.5***	***41.2***

Note: *From 12.4 $t \cdot ha^{-1}$ of vetch residues incorporated in the soil during 2015, about 3 $t \cdot ha^{-1}$ of humus will be synthesized (the humification coefficient - 0.25). The 12.4 $t \cdot ha^{-1}$ of vetch residues, incorporated in the soil, contain about 310 $kg \cdot ha^{-1}$ of nitrogen, 60% of which (180 $kg \cdot ha^{-1}$) has symbiotic origin. The ratio C: N in the dry mass of vetch is 16.5.*

The modification of the physical and chemical properties of the cambic chernozem as a result of incorporation into the soil by discussing the green fertilizers is presented in the Tab.3. The soil layers 0-35 cm being destructured and dehumificated lost resistance to compaction. The values of the penetration resistance of the investigated soil layers correlate with their apparent density values and are low for the loose layers and large for the compacted underlying strata.In the soil layer 0-10 (12) cm, as a result of the incorporation into the soil of two vetch harvest, the content of organic matter increased by 0.20%, which is a positive change in one agricultural year. It is necessary to note that this organic mass is not yet humus and represents a labile organic matter, which is easily mineralized as a result of soil microbiology processes. A trend of positive modification is also detected for other indicators of the quality status of the chernozem under the influence of green fertilizers. However, some agrochemical properties of the soil practically have not changed.

The strategic problem remains the necessity to restore mobile phosphorus content in arable soils, reducing the reserve which in the arable layer becomes catastrophic. The use of vetch green mass as an organic fertilizer solves the problem of nitrogen in the soil, but not that of phosphorus (Leah, 2015). Research data confirms that green fertilizers, solving the problem of nitrogen in the soil, lead to increase in nitrogen content, which is ecologically positive.

Table 3. Modification of the average values of the physical and chemical properties of cambic chernozem as a result of incorporation into the soil by discussing the green fertilizers

Horizon and depth, cm	Control variant (initial data)		Variant with application into the soil of one crop of vetch		Variant with application into the soil of two crops of vetch	
	Value	Assessment	Value	Assessment	Value	Assessment
Average values of apparent density, g/cm^3						
Ahp1 0-10	1.24	low	1.21	low	1.16	very low
Ahp1 10-20	1.42	high	1.42	high	1.34	moderate
Ahp2 20-35	1.53	very high	1.52	very high	1.51	veryhigh
Ah 35-50	1.43	high	1.42	high	1.43	high
Average values of the total porosity, %						
Ahp1 0-10	52.3	high	53.5	high	55.4	very high
Ahp1 10-20	45.6	moderate	45.6	moderate	48.7	moderate
Ahp2 20-35	41.8	low	42.2	low	42.6	low
Ah 35-50	46.0	moderate	46.4	moderate	46.0	moderate
Average values of resistance to penetration, kgf/cm^2						
Ahp1 0-10	13	low	11	low	9	very low
Ahp1 10-20	21	high	20	high	15	low
Ahp2 20-35	26	very high	26	very high	24	high
Ah 35-50	20	high	21	high	21	high
Content of organic matter, %						
Ahp1 0-10	3.47	moderate	3.59	moderate	3.67	moderate
Ahp1 10-20	3.33	moderate	3.30	moderate	3.37	moderate
Ahp2 20-35	3.07	moderate	3.08	moderate	3.05	moderate
Ah 35-50	2.75	submoderate	2.71	submoderate	2.76	submoderate
Content of mobile phosphorus, mg 100g^{-1} of soil						
Ahp1 0-10	1.9	moderate	2.0	moderate	2.1	moderate
Ahp1 10-20	1.4	low	1.4	low	1.3	low
Ahp2 20-35	0.8	very low	1.1	low	1.0	very low
Ah 35-50	0.8	very low	0.8	very low	0.8	very low
Content of mobile potassium, mg 100g^{-1} of soil						
Ahp 1 0-10	31	high	33	high	33	high
Ahp1 10-20	26	optimum	23	optimum	21	optimum
Ahp2 20-35	22	optimum	19	moderate	18	moderate
Ah 35-50	22	optimum	18	moderate	18	moderate
Content of nitrate nitrogen ($N\text{-}NO_3$), mg 100g^{-1} of soil						
Ahp 1 0-10	0.3	extremely low	0.6	very low	0.4	extremely low
Ahp1 10-20	0.2	extremely low	0.2	extremely low	0.1	extremely low
Ahp2 20-35	0.1	extremely low	0.1	extremely low	0.1	extremely low
Ah 35-50	0.1	extremely low	0.1	extremely low	0.1	extremely low
Content of ammonium nitrogen ($N\text{-}NH_4$), mg 100g^{-1} of soil						
Ahp 1 0-10	3.9	high	4.8	high	4.3	high
Ahp1 10-20	3.6	high	2.8	moderate	3.9	high
Ahp2 20-35	3.2	high	2.4	moderate	3.0	high
Ah 35-50	2.3	moderate	2.5	moderate	2.2	moderate

The results show that 2016 year from the point of view of atmospheric precipitation amount (329-368 mm) for the first category of crops was very favourable (Tab.4). The winter wheat harvest formed mainly from the precipitation water account, which fell during the wheat vegetation period. In the Tab.3 the data on soil humidity for control variant and variants where two harvest of vetch were introduced into the soil as a green fertilizer are presented.

Table 4. Soil moisture (%) on experimental plots

Depth, cm	Witness plot,without incorporating the vetch into the soil (2 ha)		Experimental plot, with wheat after two harvests of vetch incorporated in the soil (2 ha)	
	22.03.2016	01.07.2016	22.03.2016	01.07. 2016
0-10	23.3	25.9	23.9	25.9
10-20	23.5	23.8	25.2	23.9
20-30	24.3	23.9	26.7	25.1
30-40	25.1	23.5	26.3	24.1
40-60	24,4	23.9	26.2	23.3
60-80	24.0	20.5	24.4	19.7
80-100	23.6	20.0	23.5	19.3
0-100 (average)	*24.0*	*22.4*	*25.0*	*22.4*
Total water reserves (mm) in the 0-100 cm layer of soil in periods of winter wheat vegetation				
-	***358***	***334***	***368***	***329***
Assessment of the total water reserves in the 0-100 cm layer of soil in different periods				
-	***high***	***moderate***	***high***	***moderate***

The basic criterion for assessment the changes in the quality status of soil are the reaction of agricultural crops to these changes, which are expressed by the state of sowing and the sown crop yields. The average harvest on the control plot was 3.8 $t{\cdot}ha^{-1}$ of winter wheat. On the plot where it was incorporated into the soil by disking one harvest of vetch green mass, the wheat harvest increased by 2.4 $t{\cdot}ha^{-1}$ and made up 6.2 $t{\cdot}ha^{-1}$, and on the plot where into the soil was introduced two harvest of vetch green mass, the wheat harvest addition made up 3.2 $t{\cdot}ha^{-1}$, the total harvest of winter wheat - 7.0 $t{\cdot}ha^{-1}$ (Tab.5).

Table 5. Autumn wheat harvest on the control and experimental variants in 2016

Variant	Average wheat harvest, $t{\cdot}ha^{-1}$ (grain moisture - 8%)	Crop increase compared to the control variant	
Control (without vetch incorporation)	3.8	$t{\cdot}ha^{-1}$	%
After incorporation of one vetch green mass into the soil	6.2	2.4	63
After incorporation of two vetch green mass into the soil	7.0	3.2	84

The experimented phytotechnical method led to the remediation of the quality status only soil layer 0-10 (12) cm, to the increase of the soils production capacity and created the premises for implementation of the conservative agriculture system, based on the mini-till technology.
Soil is an organo-mineral system that can ensure a high agricultural production capacity only if there is a permanent flow of fresh organic matter in it (Lal, 2011). Creating an equilibrated or positive balance of the organic matter in the soil is the main condition for maintaining its long-term fertility and avoiding the degradation of arable layer by dehumidification, destructured and excessive secondary compaction (Leah, 2016, 2018).
This can be achieved only by the regular application into the soil of organic fertilizers - manure or green manures (Cerbari, 2015). Researches for cambic chernozem have shown the possibility to remediate the soil quality by phytotechnical methods in combination with agrotechnical ones, forming a positive balance of carbon, nitrogen and humus in the soil, stopping the degradation processes of the arable layer and regulating CO_2 emissions from the soil (Wiesmeier *et al*, 2015).
The situation in the country regarding the soil quality state at the moment can be changed only by undertaking a series of legislative, organizational, financial and phytoameliorative measures. The obtained researches, allow to recommend that within a 5-field crop rotation - one field should be introduced as a "busy field" with a sidereal leguminous: 1-2-3 harvest of vetch (or other leguminous) incorporated in the soil as green fertilizer in one agricultural year per each field of crop rotation once in 5 years. The structure of the crop rotation can be as follows: busy field, occupied with leguminous crop → maize (corn) → winter wheat → winter barley or → sunflower (Leah & Leah, 2018). This procedure, used within any system of agricultural land tillage, will lead to the formation of anequilibrated balance of organic matter in the soil, to the remediation of the quality status of the soil and to increase its production capacity.

CONCLUSIONS

The researches established that as a result of the incorporation into the arable layer of the cambic chernozem in the agricultural year 2015-2016, two green masses of vetch of about 12.4 $t \cdot ha^{-1}$ of dry matter on the "busy field" ensured: accumulation in the soil of 310 kg of nitrogen, of which 180 kg is fixed from the atmosphere; synthesis of about 3 $t \cdot ha^{-1}$ of humus or 1.7 $t \cdot ha^{-1}$ of carbon; sequestration of about 6.3 $t \cdot ha^{-1}$ of CO_2; a weakly positive balance of the organic matter and nitrogen in the soil for 3-4 years; a significant increase of wheat yield.
In the layer 0-10 (12) cm, as a result of the incorporation into the soil of two harvests of vetch, the content of labile organic matter increased by about 0.20% compared to this content in the soil on the control plot. Concomitantly, the state of physical quality of this layer was restored in a positive direction.
On the plot where in the soil was incorporated by disking, one harvest of vetch green mass the wheat yield increased by 2.4 $t \cdot ha^{-1}$ and made up 6.2 t/ha, on the plot

with twoharvest of vetch harvest, the rate made up 3.2 $t \cdot ha^{-1}$, the total harvest was 7.0 $t \cdot ha^{-1}$.

The quality of the winter wheat grains was increased, the gluten content in the wheat harvested from the plots where in the soil as green fertilizer was introduced - made up 28%, and in the wheat from the control plot - 24%.

By systemic use of green fertilizers in combination with those of phosphorus and partially of potassium, it is possible to gradually restore the state of physical, chemical and biological quality of the soils and to increase sufficiently their agricultural production capacity. The problem is to organize the system of non-polluting green fertilizers use in the agricultural sector of the Republic of Moldova and to create the seeding base for autumn and spring vetch and other leguminous cultures.

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EVALUATION OF GENETIC DIVERSITY IN SELECTED BEEF BREEDS

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ABSTRACT

The aim of the study was to estimate genetic drift and gene flow related to population structure and genetic diversity in selected beef cattle. For the evaluation of the genetic drift and gene flow among analysed populations, the Bayesian Population Structure Analysis and software Treemix were used. The genetic analysis included two cattle breeds bred in Slovakia (Charolais and Limousine). In addition to the Limousine and Charolais breeds, other beef cattle (Angus N = 90, Belgian Blue N = 4, Blonde d'Aquitaine N= 5, Hereford N = 98 and Red Angus N = 15) were analysed. The 50k Bead chip was used; the dataset consisted of 34,834 SNPs. To avoid detection bias, SNPs with high linkage disequilibrium (r^2 = 0.05) were pruned from the database; the final data set consisted of 296 animals and 2,539 SNP markers. Our results reflected four modes of gene flow between Angus, Red Angus, Charolais, Limousine and Hereford. Analysed breeds were not confirmed to influence genetic make-up of Belgian Blue and Blonde d'Aquitaine populations. All migration edges reached weight values below 0.2. The only two migration edges higher in weight was observed, first between the ancestor of Limousine breed into Blond d'Aquitaine, and second among historical ancestor of Hereford breed into Red Angus. Our results reflect that the donor population has made a significant contribution to the recipient population.

Keywords: *beef, Charolais, gene flow, Limousine, Treemix.*

INTRODUCTION

Genetic diversity is an important indicator used in improvement and conservation programs both in cattle and in other livestock populations (Hlongwane et al., 2020). Management of genetic resources in livestock populations is an important measure for maintaining the production of animal products (Makanjuola et al., 2020). Charolais and Limousine are the original French breeds of cattle, whose breeding history has 30 years tradition in Slovakia. Both breeds that were imported to Slovakia in 1990 (Kadlečík et al., 2016) belong today to the most popular breeds for use in utility crossbreeding (Association of Slovak Spotted cattle breeders -

Cooperative, 2020). Genetic diversity is an indicator of interest for both breeders and population geneticists as its status and level of variability reflect developmental processes such as adaptation, selection, gene flow and drift among populations (Goszczynski et al., 2014; Bohórquez et al., 2020). Genetic drift represents a random change in the allele frequency of an existing gene variant in a population as a result of a random sampling of organisms (Masel, 2011; Merilä, 2014). Gene flow plays a key role in the transfer of genetic diversity among populations (Bolnick and Nosil, 2007). Groeneveld et al. (2010) stated that different types of markers could be used to evaluate the state of genetic diversity or population structure, and in recent years single nucleotide polymorphism (SNP) analyses have been used in research. SNP markers are more commonly used in the analysis of genetic diversity, represent a more advantageous method due to the higher level of resolution when compared to a microsatellite marker (Vargas et al., 2016).
The aim of this study was to evaluate population structure and genetic diversity based on the estimation of gene flow and genetic drift in beef breeds bred in Slovakia.

MATERIAL AND METHODS

The SNP database consisted of 296 animals from seven cattle breeds, including two Slovak populations of Charolais (N= 67) and Limousine (N=17) cattle. The other breeds represented publicly available data of Angus (N = 90), Belgian Blue (N = 4), Blonde d'Aquitaine (N = 5), Hereford (N = 98) and Red Angus (N = 15) cattle. For Charolais and Limousine breeds, genomic DNA was extracted from the hair roots and subsequently genotyped in a commercial lab using the International Beef and Dairy (IDB) chip. The genotypic data of other breeds (Angus, Belgian Blue, Blonde d' Aquitaine, Hereford and Red Angus) were obtained using web-based data archive (McTavish et al., 2013). Quality control was performed for seven beef breeds using the PLINK v1.9 program (Chang et al., 2015), according to Kukučková et al. (2017). SNPs that reached higher linkage disequilibrium (LD) were pruned out from the database. It has been shown that pruning of SNPs with high LD counteracts the effect of the ascertainment bias and consequently makes a meaningful comparison between breeds (Kijas et al. 2009). Total of 2,539 SNPs remained for further analysis. Population structure among beef populations was estimated by Bayesian Population Structure Analysis (BAPS) version 6.0 (Corander and Tang, 2007). According to Kukučková et al. (2017) were set the number of cluster K 7-50 and 1000 simulations from the posterior allele frequencies. For recognition of genetic drift and gene flow among populations were used the Treemix program (Pickrell and Pritchard, 2012). First, a phylogenetic tree of evaluated bovine populations was created, which was based on maximum probability, and then migration edges (m = 8) were added to the generated graph (Upadhyay et al., 2019). All graphs were visualised using the statistical software R (R Core Team, 2014).

RESULT AND DISCUSSION

Based on our results, it is possible to see the occurrence of gene flow between populations (Figure 1). Arrows point on the specific populations in the graph, expressing the given degree of admixture of individual populations. Tang et al. (2009) reported that a typical population, in addition to own primary resources, is mostly made up of small portions of gene flow from other populations. The observed level of admixture in this study was expressed in the graph as arrows between populations, with the corresponding numbers being the result of random generation from a uniform distribution (Kukučková et al., 2017). Our results reflect four modes of gene flow between Angus (cluster 1), Red Angus (cluster 7), Charolais (cluster 4), Limousine (cluster 6) and Hereford (cluster 5). The gene flow between the above breeds expressed by the fact that clusters 1, 4, 5, 6 and 7 separately represent a source of migrants but at the same time accept the flow of genes from other populations. The gene pools of Belgian Blue (cluster 2) and Blonde d'Aquitaine (cluster 3) were not affected by other breeds under consideration, and therefore they did not show four modes of gene flow. Figure 1 illustrates their contribution into the genomes of Red Angus, Angus, Charollais, Limousin and Hereford.

The Slovak populations of Charolais and Limousine achieved a relatively high proportion of genotypes that have sequence signatures from other clusters, which indicates extensive migration events between these populations. The inter-clusters gene flow ranged from 0.03% to 8.90%. The analysis of gene flow showed that only 2% of the Charolais genome was affected by other breeds. The majority of genes come from Limousine and Blonde d'Aquitaine cattle, with a contribution of 0.79% and 0.42%, respectively.

The Limousine genome showed a higher value of gene flow compared to Charolais, where up to 5% of introduced DNA were obtained from other populations. The two significant sources of gene flow come from Charolais and Blonde d'Aquitaine, with a contribution of 3.50% and 0.82%, respectively.

Belgian Blue and Blonde d'Aquitaine did not show a significant level of gene flow from the other clusters. The Angus had a higher gene flow from the Charolais (1.20%) compared to the Limousine (0.43%). Red Angus had the highest gene flow from the Angus (8.90%) as expected. On the contrary, Hereford had the main source of gene flow from Charolais (1.10%).

Our results show that the current breeding standard of the Charolais and Limousine breeds is the result of historical cooperation in the formation of these breeds. According to the results from Figure 1, the Limousine and Charolais breed did not participate in the formation of the Blonde d'Aquitaine breed, respectively, indicate that its gene pool was the only rarely influenced by other breeds. However, we know from history that the Southwestern populations of French cattle, including the Limousine, participated in the formation of the Blonde d'Aquitaine breed. Ritchie (2009) confirm our results, as there are historical branches between the Blonde d'Aquitaine and Charolais. Since can be seen from the results that the Blonde d'Aquitaine occurred in Limousine and Charolais clusters as the two major sources of gene flow, we can say that there is an exchange between the given populations. We believe that the gene flow for the Blonde d'Aquitaine is less than 0,18%, and therefore, the program does not display it. Ouchene-Khelifi et al. (2018) confirm our opinion when they state that gene flow with a value lower than 0.01 was not displayed due to pruning to improve the readability of the images.

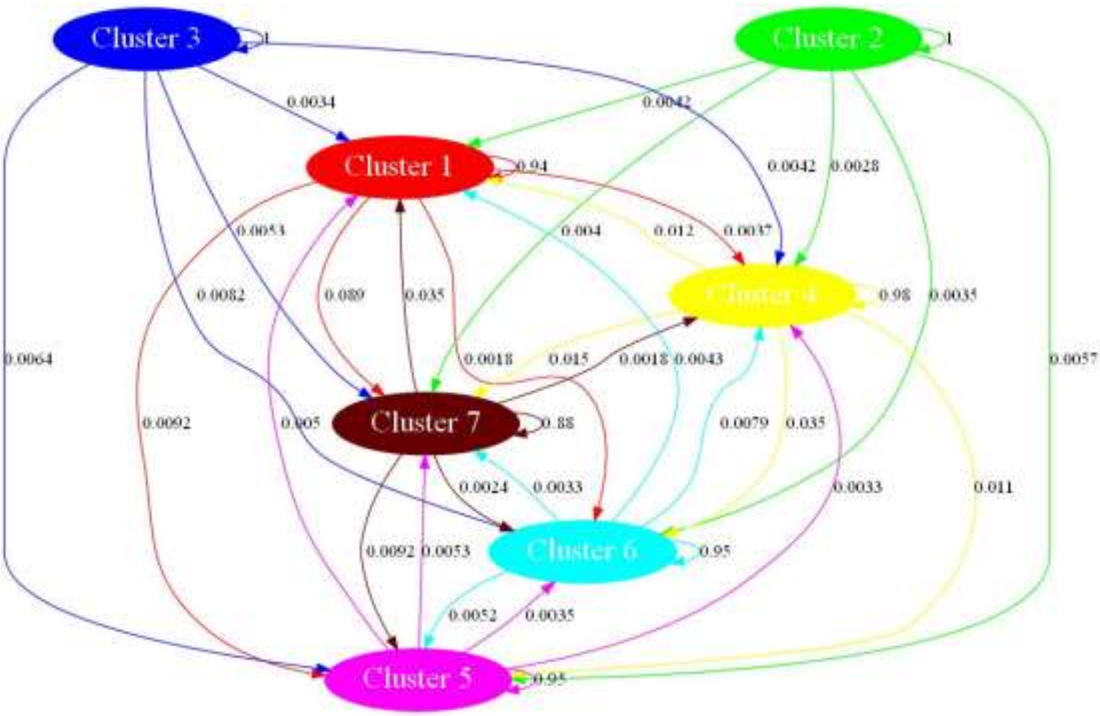

Figure 1. The gene flow graph in evaluated beef populations. (Angus = Cluster 1, Belgian Blue = Cluster 2, Blonde d'Aquitaine– Cluster 3, Charolais – Cluster 4, Hereford – Cluster 5, Limousine – Cluster 6 and Red Angus – Cluster 7)

Based on our results was created the maximum-likelihood (ML) phylogenetic ancestry graph expressing gene flow edges among populations. The results indicated the formation of three groups (Figures 2), but the distribution of the individual populations was slightly different compared to Bayesian analysis. Figure 2. represents the ML tree for seven evaluated beef populations, including migration arrows. Migration arrows are coloured according to the weight and the degree of admixture estimated among the evaluated breeds. The amount of gene flow between populations was expressed using a horizontal axis scale and individual horizontal branches. The standard error was stated as 10x the average standard error of the covariance matrix of a given sample. All breeds showed relatively long branches in the ML graph, except the Charolais, Belgian Blue and Blonde d'Aquitaine (Figure 2), indicating that these breeds were carried away similarly as reported by Rochus et al. (2017). It can be seen that the Limousine and Blonde d'Aquitaine are more genetically linked and, besides, appeared on the same branch. The results point to the fact that the Limousine was involved in the formation of Blonde d'Aquitaine and have common historical ancestors. As expected, the Angus and Red Angus are more genetically linked together and appeared on the same branch as the Hereford breed similarly as reported by Barbato et al. (2020). The Charolais breed is separated from other breeds under consideration.

Figure 2 shows that the Charolais and Blonde d'Aquitaine are genetically linked, occurring on the same branch as the Limousine. After the addition of migration events, we observed the influence of Limousine on Blonde d'Aquitaine, which suggests that introgressive events have historically occurred between these breeds, which is consistent with the results of BAPS analysis. When migration events were observed, the average migration edges had a weight less than 0.2, the only exception being the edge linking the ancestors' Limousine and Blonde d'Aquitaine. This migration event reached a value higher than 0.4, indicating that the donor population contributes significantly to the recipient population as reported by Orozco-ter Wengel et al. (2015). The Charolais had a low weight of the migration edge from the Red Angus (0.2) and vice-versa. Our results most likely pointed to a historical crossbreeding to create a hybrid of these two breeds (Keane, 2011) with high carcass and performance traits (Nelson, 2015).

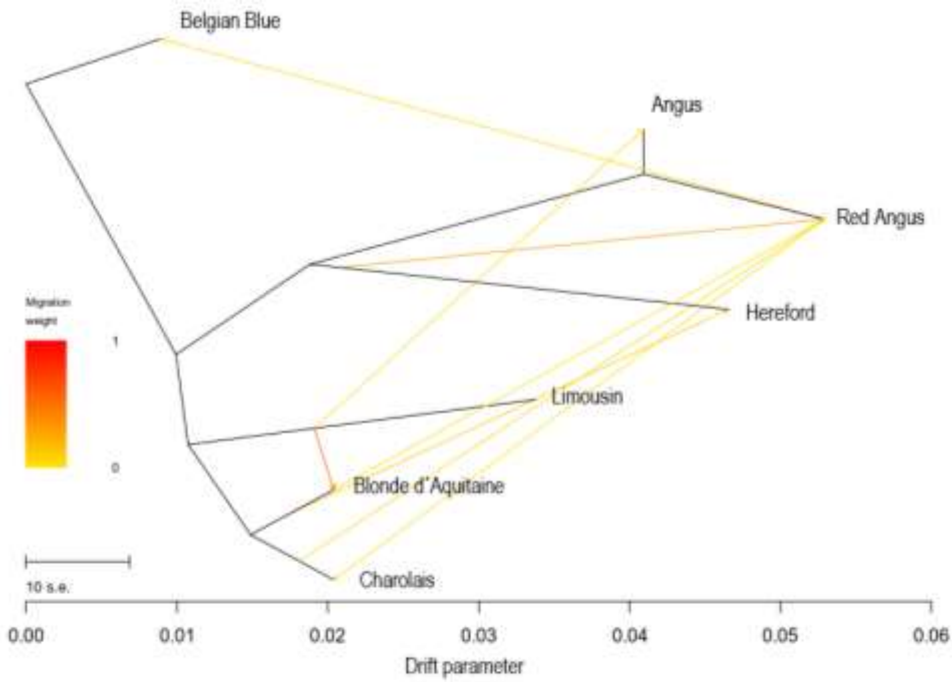

Figure 3. The maximum-likelihood (ML) tree represented the inferred relationship between analysed beef populations (with migrations settings)

CONCLUSION

The current state of modern breeds genomes is the result of lengthy and complex processes. With the help of new advanced analyses, it is possible to reveal these processes that have been involved in the formation of current cattle breeds. Obtained results indicated that the Limousine genome was influenced mainly by the Charolais genes and vice-versa. Both applied methodologies confirmed the historical contribution of Charolais cattle to the genetic make-up of Red Angus. Moreover, the Charolais genome showed genetic signatures of crossbreeding with Blonde d'Aquitaine in the 60s of the 19th century. In general, the results of this study provided knowledge about the degree of gene flow and genetic admixture among evaluated breeds, mainly due to migration events and crossbreeding during grading-up of the particular breeds. Future genome-wide association studies involving haplotypes unique for each breed can be a good basis for genomic improvement of specific carcass and performance traits.

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LONG-TERM RELATIONSHIP BETWEEN OAK DECLINE AND SHRUB GROWTH DYNAMICS IN AN HUNGARIAN OAK FOREST, 1972-2017

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ABSTRACT

Long-term structural dynamics of shrub layer of temperate oak forest communities were not extensively reported in published studies. The serious oak decline was first reported in 1979-80 and nowadays 63.0% of canopy oak trees died in a forest stand. The data were used to obtain (1) quantitative information on shrub layer growth, including height (H) and shoot diameter (DSH) condition and basal area (BA) values; (2) structural information on foliage cover rate of the shrub layer, mean cover of some shrub species; (3) comprehensive description from the ecological processes in the shrub layer in the last 45 years and our objective was (4) to analyze the possible effects of oak decline on the shrub growth dynamics. The following measurements were carried out in the 48 × 48 m plot: shoot height, shoot diameter, basal area and foliage cover of each individuals in the high shrub layer. Correlation analysis confirmed that significant positive relations were between mean H, mean DSH of the dominant woody species (*Acer campestre*, *Acer tataricum* and *Cornus mas*) and oak tree density between 1972 and 2017. The decreasing oak tree density did not show detectable impact to the co-dominant shrubs growth. There was a low significant association between number of oak trees and basal area of high shrub layer. Finally, there was a statistically significant interaction between mean cover of *A. campestre* and *C. mas* and oak trees. The findings of the study indicate that forest responded to oak decline with significant structural rearrangement in the shrub layer.

Keywords: *Acer campestre, understory, height, diameter, mean foliage cover*.

INTRODUCTION

Shrub layers of forests change dynamically and respond sensitively to the environmental changes (Chipman and Johnson, 2002; Rees and Juday, 2002). They are strongly related to the structure and composition of the tree layer (De Grandpré *et al*., 1993; Klinka *et al*., 1996). Understory plants are important components of forests because shrubs play a crucial role in the cycles of some essential nutrients, including the dynamics of N, K and carbon (Gilliam, 2007). The shrub layers of forests are directly contributes to the forest biodiversity (Kerns and Ohmann, 2004; Aubin *et al*., 2009), enhancing the aesthetics of forest ecosystems and helping to protect watersheds from erosion (Alaback and Herman, 1988; Muir *et al*., 2002). Shrubs can mitigate forest decline and influence forest regeneration through affecting light availability (Kunstler *et al*., 2006). On the other hand, tree and shrub individuals may compete for resources such as light, nutrients, or water during later stages of development (Wang et al., 2016). The shrub's cover may also vary along with the changes in tree density (Hallinger *et al*., 2010).

Serious oak decline was first reported in 1979–80 from our study site, heavily affecting *Quercus petraea* Matt. L. (sessile oak) individuals, and by 2017, 62.9% of canopy oak trees had died (from 816 living trees to 303 trees ha^{-1}). An increase in the decline of living oak trees was reported in many regions of Hungary since 1978 (Kotroczó *et al*., 2007). Many biotic and abiotic factors have been identified as important in oak decline events, such as extreme weather conditions (Drobyshev *et al*., 2008; Bolte *et al*., 2010), insect fluctuations (Moraal and Hilszczanski, 2000), disease outbreaks (Mistretta, 2002) or climate change, air pollution and fires (Signell *et al*., 2005; Kabrick *et al*., 2008). The resulting changes in the forest stand were described in many papers (Jakucs, 1988; Kotroczó *et al*., 2005; Mészáros *et al*., 2011; Misik *et al*., 2014, 2017).

Few published papers have investigated the long-term dynamics and structural changes in the understory shrub layer of deciduous oak forests (Alaback and Herman, 1988; Chapman *et al*., 2006; Gracia *et al*., 2007; Gazol and Ibáñez, 2009; Chapman and McEwan, 2016). Our comprehensive investigations play a gap-filling role.

The research data were used to obtain (1) quantitative information on shrub layer growth, including height (H) and shoot diameter (DSH) condition and basal area (BA) values relation with oak tree density; (2) structural information on foliage cover rate of the shrub layer, mean cover of some shrub species relation with oak tree density; (3) comprehensive description from the ecological processes in the shrub layer in the last 45 years and our objective was (4) to analyse the possible effects of oak decline on the shrub growth dynamics.

MATERIAL AND METHODS

The 24ha reserve study area is located in the Bükk Mountains of northeast Hungary (47°55′ N, 20°46′ E) and at an altitude of 320–340 m above sea level. Descriptions of the geographic, climatic parameters, soil conditions, and vegetation of the forest were reported in detail by Jakucs (1985, 1988). The most common deciduous forest association in this region is *Quercetum petraeae-cerridis* Soó 1963 (sessile oak−Turkey oak) forest with a dominant canopy of *Q. petraea* and *Quercus cerris* L. (Turkey oak). Both oak species are important dominant, native tree in Hungarian natural woodlands.

Monitoring activities started in 1972 and it was repeated in 1982, 1988, 1993, 1997, 2002, 2007, 2012 and finally in 2017 in the growing seasons. The shrub layer was divided into a low and a high sub-layer in the 48 × 48 m monitoring plot.

Specimens which were higher than 1.0 m were categorized as high shrubs. Lower specimens were categorized as low shrubs. The term "dominant woody species" is used to refer to the *Acer campestre* L. (field maple), *Acer tataricum* L. (Tatar maple) and *Cornus mas* L. (European cornel) that play a key role in the understory. Several size variables of each high shrub specimen in the sampling plot were determined. Plant height (H) was measured with a scaled pole and shoot diameter (DSH) at 5.0 cm above the ground with a digital caliper. Total basal area of the high shrub layer and of high shrub species was calculated based on the shoot diameter values (BA, m^2 ha^{-1}). Mean cover of high shrub species and actual foliage cover of high shrub layer were calculated in m^2 and in the latter case expressed in percentage of the permanent sampling area. The foliage map was built in a GIS environment (ESRI, 1999). Based on the digitized map we estimated the foliage cover values with the Spatial Analysis Tools - Calculate Area function of the GIS. Statistical regression analysis was performed using the PAST statistical software and significant differences for all statistical tests were evaluated at the level of $^{*}P < 0.05$; $^{**}P \leq 0.01$. There was no significant correlation found between the test variables at $^{n.s.}P \geq 0.05$.

RESULTS AND DISCUSSION

Mean height and diameter, basal area of shrub species and of shrub layer, foliage cover and other importance values of understory shrub layer are given in Table 1 and in Table 2. Mean H of dominant woody species in the shrub layer increased considerably after the start of the oak decline; these species reached maximum below 3.0 m in height before the oak decline and were growing suddenly after 1982 and were measured between 5.3-8.7 m in height to 2017. Mean H of the co-dominant shrubs increased from 1.8 m to 2.4 m until 1997, after which it started to decrease again. Mean DSH of these species increased from 1.5 cm to 2.9 cm; however, after 1993, the mean values started to decrease. BA of the understory was only 0.005 m^2 ha^{-1}. After the decline, already in 1982, a considerably increase in the high shrub layer's BA was found, and this continued in the following observed years. The biggest total BA was recorded in the second last measuring with 11.66 $m^2 \cdot ha^{-1}$. The rate of maples species and *C. mas* BA together in the total BA was higher than 89.0%.

Table 1. Long-term tendency of the mean size values (±SD) in the high shrub layer.

year	height (m)				diameter (cm)			
	A. camp.	*A. tatar.*	*C. mas*	co-dom. shrubs	*A. camp.*	*A. tatar.*	*C. mas*	co-dom. shrubs
1972	2.29	2.68	2.36	1.75	2.60	2.41	2.45	1.48
1982	4.83	3.43	3.64	2.21	5.20	3.39	3.95	1.91
1988	4.85	3.52	3.69	2.25	6.11	3.57	4.44	2.36
1993	5.20	3.37	3.81	2.34	6.63	4.69	5.68	2.92
1997	5.21	3.75	3.87	2.37	6.83	4.63	4.89	2.46
2002	5.88	4.22	4.66	2.14	8.61	5.36	6.43	2.33
2007	8.23	4.92	4.85	1.96	11.03	6.45	7.82	2.19
2012	7.60	5.50	5.37	1.82	10.63	7.4	7.50	1.94
2017	8.74	5.31	5.26	1.86	9.61	6.08	6.95	2.00
mean±SD	5.87 ±2.02	4.08 ±0.97	4.17 ±0.96	2.08 ±0.23	7.47 ±2.75	4.89 ±1.61	5.57 ±1.79	2.18 ±0.41

The regression analysis confirmed that significant positive relations were between mean H of maples species and decreasing oak tree density (r = 0.77* and 0.72*) between 1972-2017. This relation between canopy tree density and mean H of *C. mas* (r = 0.82**) and mean DSH of dominant woody species (r = 0.84**, 0.80**, 0.84**) was stronger (Fig. 1. A, B). The relationship was non-significant between oak density and mean sizes of co-dominant shrub species ($r = 0.25^{n.s.}$ and $0.62^{n.s.}$).

Table 2. Long-term tendency of the mean cover, foliage canopy and basal area values (±SD) in the high shrub layer.

year	mean cover (m^2)			foliage canopy (%)	basal area (m^2 ha^{-1})			
	A. camp.	*A. tatar.*	*C. mas*		*A. camp.*	*A. tatar.*	*C. mas*	high shrub layer
1972	2.79	1.87	2.45	64.40	0.17^{-2}	0.13^{-2}	0.13^{-2}	0.48^{-2}
1982	4.06	3.19	4.14	85.30	1.85	0.30	0.90	3.13
1988	5.59	3.32	5.21	84.16	2.18	0.19	0.89	3.40
1993	6.88	4.47	7.93	74.00	1.97	0.28	0.91	3.49
1997	7.23	3.20	5.66	79.50	3.00	0.34	1.38	5.30
2002	6.22	5.58	7.18	67.50	4.19	0.42	1.54	6.85
2007	11.54	9.71	12.44	86.20	4.69	0.35	2.18	7.99
2012	6.12	4.29	6.39	61.48	6.35	0.57	3.92	11.66
2017	12.34	9.97	12.67	91.26	5.16	0.31	1.66	8.04
mean±SD	6.97 ±3.14	5.07 ±2.90	7.12 ±3.48	77.09 ±10.68	3.27 ±1.99	0.31 ±0.16	1.49 ±1.10	5.54 ±3.48

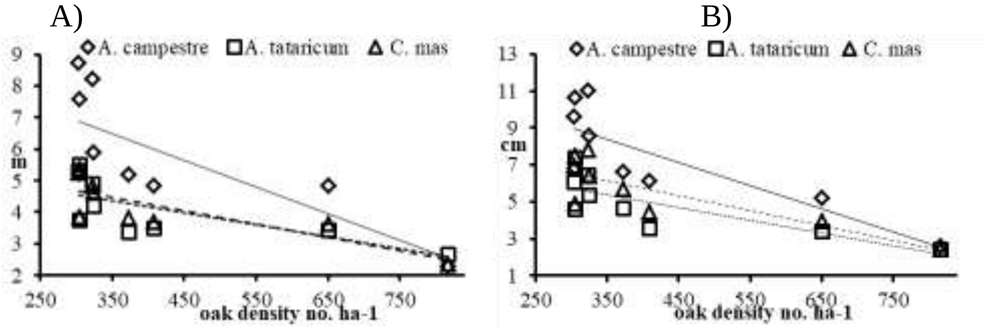

Figure 1. Relationship between oak tree density and (A) mean height, (B) mean diameter of the dominant woody species between 1972–2017. [*A. campestre* ––. (A) $R^2 = 0.60$, $P < 0.05$; (B) $R^2 = 0.70$, $P \leq 0.01$; *A. tataricum* ⋯, (A) $R^2 = 0.51$, $P < 0.05$; (B) $R^2 = 0.64$, $P \leq 0.01$; *C. mas* - - - , (A) $R^2 = 0.68$, $P \leq 0.01$; (B) $R^2 = 0.70$, $P \leq 0.01$]

The analysis did show a significant relation for maples species (r = 0.79*, 0.74*), for *E. verrucosus* (r = 0.75*) and for high shrub community (r = 0.77*) between BA values and decreasing oak tree density (Fig. 2. A). Over last 45 years; the association is non-significant for BA of *C. mas* ($r = 0.65^{n.s.}$). Low significant relationship are observed between mean cover of *A. campestre, C. mas* and oaks density (r = 0.70* and 0.68*) (Fig. 2. B). Changes of mean cover of *A. tataricum* ($r = 0.57^{n.s.}$), co-dominant shrubs ($r = 0.58^{n.s.}$), foliage cover of the high shrub layer ($r = 0.20^{n.s.}$) and oak decline for the long-term study are found to have a non-significant relationship. According to Röhrig and Ulrich (1991) *A. campestre* is a relatively drought tolerant species. On the other hand, maples have got "Oskar"-strategy (Silvertown, 1982). Maples typically develop a “sit-and-wait” strategy so they wait for example for the canopy decline events. Oaks cannot successfully compete with these species (McDonald *et al*., 2002, Zaczek *et al*., 2002). Our results support these statements, because in our site maples showed a significant increase in size and foliage cover after the oak decline. In the upland oak forest of USA the total basal area in the understorey was substantially higher in 2002 than in 1934, increasing from 0.9 to 3.6 $m^2 \cdot ha^{-1}$, while the density of most oaks and shortleaf pines in the canopy decreased (Chapman *et al*., 2006). We found similar tendency in Síkfőkút. Our findings confirm that long-term DSH changes of the dominant woody species and size values of *A. campestre* most significantly associated with oak decline (Fig. 1., 2.).

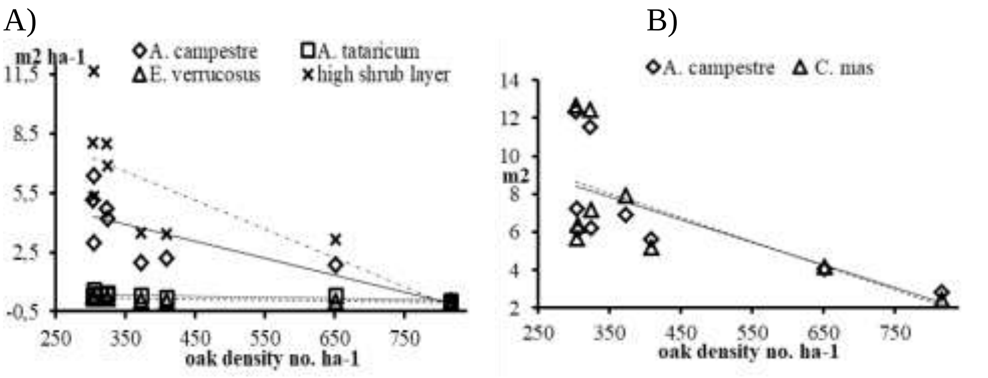

Figure 2. Relationship between oak tree density and (A) basal area, (B) foliage cover changes in the shrub layer between 1972–2017. [*A. campestre* —. (A) R^2 = 0.63, P < 0.05; (B) R^2 = 0.49, P < 0.05; *A. tataricum* ···, (A) R^2 = 0.55, P < 0.05; *E. verrucosus* - - - , (A) R^2 = 0.56, P < 0.05; high shrub layer -•-•- (A) R^2 = 0.60, P < 0.05; *C. mas* - - - , (B) R^2 = 0.46, P < 0.05]

CONCLUSION

Our study suggests that (1) dominant woody species growth was significantly affected by serious oak decline; this association was higher to the DSH values of these species. (2) Decreasing density of canopy oak trees was significantly affected on the long-term trend of basal area of maples species and *E. verrucosus*. The association was similar to the high shrub community. (3) A significant relationship between mean cover of *A. campestre* and *C. mas* and oak tree density was observed for the 45 years in the studied forest stand. Overall, the shrub layer condition and growth dynamics consistently associated with canopy oak mortality.

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COMPLEX ASSESSMENT OF BIOSOLID FOR AGRICULTURE USING LIVING ORGANISMS UNDER LABORATORY CONDITIONS

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ABSTRACT

The use of biosolids (treated municipal sewage sludge) as a fertilizer is the best way of their disposal. However, not all of them are suitable for use as a fertilizer. Biosolids should be subject to mandatory laboratory control to confirm their safety. Two directions of research on biosolids are being improved: chemical and biological. Chemical analysis methods allow us to determine the qualitative composition of complex waste. The biological approach (use of living organisms) allows us to estimate the total toxicity of all the components. Accordingly, a distinctive characteristic of biological methods is the integrated approach. We examined biosolid extract using a wide range of bioassay methods. As test organisms, we took *Daphnia magna* Straus, *Paramecium caudatum* Ehrenberg, *Tetrahymena pyriformis*, luminescent bacteria *Escherichia coli*. In addition, a phytotest was carried out on the culture of *Avena sativa* L. and *Raphanus sativus* L. None of the tests revealed a high toxicity of biosolid. Biosolid safety was confirmed by a low content of potentially toxic water-soluble elements – (μg /l): Al^{3+} – 980; Ba^{2+} – 19; B – 140; Mn – 360; Cu – 61; As – 57; Ni – 200; Pb – 1,4; Sr^{2+} – 302; Cr – 18; Zn^{2+} – 310; Co – 30; Mo – 56; (mg/l): Na^{+} – 16,8; Fe – 1,0. The bioassay methods make it possible to give an indicative safety assessment of this type of object by the effect of readily soluble (readily available) components from this object on living organisms and plants. The use of bioassay methods using soil extraction as a control tool allows to take into account the combined effect of the presence in the extraction of not only toxic elements that suppress the vital activity of organisms, but also of elements that attract and stimulate the activity of test-organisms.

Keywords: *bioassay, biosolid, sewage sludge, environmental safety, agriculture.*

INTRODUCTION

Continuously forming municipal sewage sludge (SS) is not only waste, it can also become a valuable resource for agriculture due to its similarity with traditional organic fertilizers on the composition of nutrient elements and organic substance.

However, the concomitant presence of heavy metals, organic pollutants (polychlorinated hydrocarbons, polycyclic hydrocarbons, pesticides, pharmaceuticals, including hormones and antibiotics) and pathogenic microorganisms becomes a significant limiting factor in their use as a fertilizer (Smith, 2009; Clarke, 2011; Delibacak, 2020). SS safety for agriculture (AC) is defined by the results of chemical analysis (Collivignarelli, 2019). However, the discrepancy in the regulation of chemicals, the inability to set an exact vector in the search for all toxic components makes it difficult to adequately assess the safety of the given object. Within this context, biological methods being integral methods of assessing toxicity are gaining great demand (Lyashenko, 2012; Zhmur, 2012). Biological methods (or bioassays) are a set of simple inexpensive and relatively fast in their implementation methods. With their help it is possible to identify the contribution of toxic components unaccounted by chemical and to give a primary integral assessment of the influence of all water-soluble components present in SS on living organisms (Gelashvili, 2016). The lack of a clear protocol on SS research using biological methods dictates the necessity in the development of the given direction. In this regard, we set a goal to estimate the approaches and methods of bioassay of waste water sludge processing products for their safe use in agriculture.

MATERIAL AND METHODS

The product of processing (compost) of aerobic-stabilized sewage sludge is studied in the work. The sewage sludge formed in Vladimir (Russia) (56.1428° N, 40.3896° E) treatment facilities was subjected to a biennium mesophilic composting with sawdust of coniferous trees in the ratio of 1:0.8 without the use of microbiological preparations. The compost represented a homogeneous mass of dark gray color (Figure 1). Determination of the total amount, potentially active forms (ammonium acetate buffer – AAB, pH=4.8), water-soluble forms of elements (water extract in the ratio of distilled water being 1:1) were performed in the water extract of compost using inductively coupled plasma mass spectrometry (ICP-MS). Bioassay (including phyto-testing) was performed using a wide range of test organisms of various trophic groups. The samples were examined using *Daphnia magna* Straus, *Paramecium claudatum* Ehrenberg, *Tetrahymena pyriformis*, an «Ecolume» biosensor based on a luminescent strain of *Escherichia coli, Avena sativa* L., *Raphanus sativus* L. Table 1 provides a brief characteristic of each method with of test organisms' toxicity indicators and criteria. An extraction (pH=7,2) of uncontaminated soil – Umbric ALBELUVISOLS (WRB) (the upper 20 cm of soil layer) formed on the binomial glacial deposits the plough layer of which is thicker loamy sediments overlying heavy moraine loam was used as a control for bioassay apart from the provided methods of distilled water or culture medium. SS and its processed products are local fertilizers and their use is confined to this type of soils. Extractions for bioassay are prepared in the following ratio compost (soil): distilled water – 1:1, 1: 10. In domestic guidelines, it is mainly recommended to use an extract of 1: 10 for waste and 1:5 for soil in a ratio with distilled water. In order to avoid breeding errors, we studied the extraction of compost and soil 1:1.

Repeatability for each test takes three times. Statistical processing of results was performed using MS Excel and STATISTICA 6.1 programs in accordance with the guidelines.

Figure 1. Compost of aerobic-stabilized sewage sludge

Table 1. Brief characteristic of bioassay methods used in the work.

Bioassay method	Test-organism	Toxicity indicator/criterion
Determination of the toxicity of water extractions by the survival rate of *Daphnia* (multicellular organism)	*Daphnia magna* Straus	Death of test organisms in 48 hours ≥50%
Determination of toxicity of water extractions by survival and chemotactic reaction of infusoria (single-celled organism)	*Paramecium caudatum* Ehrenberg	Decrease of chemotactic response (chemotaxis) *Paramecium caudatum* Ehrenberg/≥40%
Determination of the toxicity of water extractions by the growth function of infusoria (single-celled organism)	*Tetrahymena pyriformis*	Augmentation decrease of *Tetrahymena pyriformis* cells in 48 hours /≥50%
Determination of the toxicity of water extractions by the glow activity of lyophilized fluorescent bacteria *Escherichia coli*	“Ecolume” biosensor (luminescent bacteria *Escherichia coli*)	Suppression of bacterial bioluminescence intensity for a 5-30-minute exposure period /≥20%
Phyto-testing (eluated method)	*Avena sativa* L., *Raphanus sativus* L.	Suppression of root length of seedlings /≥20%

RESULTS AND DISCUSSION

The study of the chemical composition of complex multicomponent objects is mandatory, at that special attention should be paid to the form of finding the detected components. Knowing their form we can assume its behavior under various conditions. Table 2 represents the content of several components in the total amount and potentially active form of elements.

Table 2. The total content and the content of potentially active form.of elements in compost

Element	Total amount	Potentially active form – AAB (pH=4,8)
mg/kg of dry solid		
Li	3,8 ± 0,6	-
Be	1,2 ± 0,2	-
B	17,0 ± 2,6	-
Na	430,0 ± 65,0	-
Mg	5620,0 ± 840,0	267,0 ± 32,0
Al	14000,0 ±2100,0	31,0± 4,0
Ti	79,0 ± 12,0	-
V	22,0 ± 3,3	-
Cr	82,0 ± 12,0 (–/90-500)*	-
Mn	440,0 ± 66,0	34,0 ± 0,4
Fe	14000,0 ± 2100,0	39,0 ± 4,7
Co	5,2 ± 0,8	0,1 ±0,01
Ni	37,0 ± 5,6 (300-400/80-200)	1,0 ± 0,2
Cu	240,0 ± 36,0 (1000-1750/132-750)	0,6 ± 0,04
Zn	540,0 ± 81,0 (2500-4000/220-1750)	-
Ga	11,0 ± 1,7	-
As	5,0 ± 0,8 (–/2-10)	-
Se	0,7 ± 0,1	-
Br	640,0 ± 96,0	-
Rb	9,4 ± 1,4	-
Sr	100,0± 15,0	14,0 ±1,4
Cd	1,6± 0,2 (20-40/2-15)	0,1 ± 0,02
Sn	5,6 ± 0,8	-
W	1,3 ± 0,2	-
Pb	22,0 ± 3,3 (750-1200/130-250)	1,6±0,2
Ba	230,0 ± 35,0	19,0 ± 2,5
Zr	13,0± 2,0	-
Ag	3,5 ± 0,5	-
Th	1,0 ± 0,2	-
U	4,6 ± 0,7	-

La	5,5 ± 0,8	-
Ce	11,0 ± 1,7	-
Pr	1,2 ± 0,2	-
Nd	4,3 ± 0,7	-
ppb		
Hg	590 ± 89 (16000-25000/2100-7500)	-
Sb	350 ± 53	-
Mo	3,4 ± 0,5	-
Yb	270 ± 41	-
Hf	300 ± 45	-
Ta	14 ± 2	-
Tl	68 ± 10	-
Ge	50 ± 8	-
Nb	75 ± 11	-
Te	28 ± 4	-
Cs	560 ± 84	-
Sm	820 ± 120	-
Eu	240 ± 36	-
Gd	860 ± 130	-
Dy	580 ± 87	-
Ho	110 ± 17	-
Er	310 ± 47	-
Tm	43 ± 7	-

*() – concentration limits for heavy metals in sludge according to Directive 86/278/EEC / Russian State standard

The content of heavy metals in the test compost according to Directive 86/278/EEC does not exceed the limits. However, according to national standards that have stricter standards, compost is not recommended to be used for growing forage, technical, grain crops, green manures and for growing seedlings of vegetable and flower crops on a personal household nonetheless it is suitable for planting forestry crops along roads, in nurseries of forest and ornamental crops, floriculture, for cultivating depleted soils, recultivation of disturbed land and slopes of motor roads and recultivation of solid waste dumps (National Standard GOST R 54651-2011).

It should be noted that elements the presence of which isn't standardized and reaches tens of mg/kg are found in the test compost. Whereby the role of some of them hasn't yet been completely studied. Their presence can affect the total estimation of toxicity. Table 3 represents the values of content of water-soluble elements that pass into the extraction 1:1.

Table 3. The content of several elements of water extraction of compost (1:1).

Element	Mean value
mg/l	
Na^{+}	16,8
Fe	1,0
μg /l	
Al^{3+}	980
Ba^{2+}	19
B	140
Mn	360
Cu	61
As	57
Ni	200
Pb	1,4
Sr^{2+}	302
Cr	18
Zn^{2+}	310
Co	30
Mo	56

The content of elements in the compost extraction doesn’t exceed the limits stipulated in the national standards for the content of adverse elements in drinking water (Sanitary Rules and Regulations SanPiN 2.1.4.1074-01).
According to the results of bioassay, *Daphnia magna* Straus test-organisms (Figure 2a, Table 4) showed 100% survival in all the variants at 48-hour exposure which can reveal the absence of acute toxicity of compost extractions on the basis of sewage sludge for the given test.
According to the influence of water extractions of compost on the change in the intensity of bioluminescence of bacteria (biosensor "Ecolume", represented by luminescent bacteria *Escherichia coli*), no acute toxic effects were detected as well (Table 4).

a b

Figure 2. Daphnia magna Straus (a), Paramecium caudatum Ehrenberg (b)

The components that are most sensitive to sewage sludge turned out to be organisms belonging to the same type: *Paramecium caudatum* (Figure 2b) and *Tetrahymena pyriformis* infusoria. As can be seen from Table 4 regarding the Lozina-Lozinsky medium provided by the method (*Paramecium caudatum*), all the extractions did not have a high acute toxicity (2% and 13%). As for soil extractions, compost extractions are less attractive and a significant increase in the percentage of toxicity (67% and 46%) indicates it.

Table 4. Compost toxicity in regard to various test-organisms, %.

Compost extraction	In regard to control	In regard to soil extraction
	Daphnia magna Straus	
Compost extraction 1:1	0	0
Compost extraction 1:10	0	0
luminescent bacteria *Escherichia coli*		
Compost extraction 1:1	0	0
Compost extraction 1:10	0	0
Paramecium caudatum Ehrenberg		
Compost extraction 1:1	2	67
Compost extraction 1:10	13	46
Tetrahymena pyriformis		
Compost extraction 1:1	13	7
Compost extraction 1:10	-19	-5
Avena sativa L.		

Compost extraction 1:1	17	26
Compost extraction 1:10	-17	-14
Raphanus sativus L.		
Compost extraction 1:1	13	20
Compost extraction 1:10	-14	-13

*symbol "-" stands for stimulation

Dolgov's researches have shown no toxicity of compost extractions based on sewage sludge with minimal dilution with distilled water (1:1) for augmentation of *Tetrahymena pyriformis* cells (Dolgov, 2014). According to the results of our studies presented in table 4, according to the influence of compost extractions based on sewage sludge on the augmentation of *Tetrahymena pyriformis* cells, the absence of their acute toxicity can be noted. Moreover, the extraction of compost 1:10 had a stimulating effect on the augmentation of infusoria cells. It is important to emphasize that by 48 hours, there was an inhibition of the augmentation of infusoria cells under the influence of extraction 1:1, that constituted 13%. In reference to soil extracts, the effect was somewhat lower.

A number of authors have identified the phytotoxicity of compost extractions based on sewage sludge on various crops (Mitelut, 2011; Adamcová, 2016). During phytotesting on barley (*Hordeum vulgare* L.) and garden-cress (*Lepidium sativum* L.) the author Fuentes, detected no toxicity, which may be due to a low content of easily soluble toxic components (Fuentes, 2004).

Table 4 also shows the results of testing compost extractions based on sewage sludge to detect an inhibitory effect on the germination of roots of monocotyledonous and dicotyledonous crops. Low inhibition of root growth of *Avena sativa* L. and *Raphanus sativus* L. was detected (13% and 20%). A stimulating effect was noted in the study of the extraction 1:10.

The use of bioassay methods using soil extraction as a control tool allows us to take into account the combined effect of the presence in the extraction of not only toxic elements that suppress the vital activity of organisms, but also of elements that attract and stimulate the activity of test-organisms. There are following quantities of important components for plants: N total – 2,6±0,2%, P_2O_5 – 8,45±0,3%, K_2O – 0,51±0,05%, Ca total – 24 g/kg. However, the choice of control in each particular case requires a clear scientific and practical justification.

CONCLUSIONS

Biotesting along with chemical analysis is an important component of the study of complex multicomponent objects. The use of chemical composition is not sufficient for determining the total toxicity due to the inability to set an exact vector in the determination of all the toxic components. Bioassay methods enable to detect or confirm the absence of toxicity of all active substances present. The use of water extractions can not only reveal the toxicity of sewage sludge and waste products, but also enable to estimate the possibility of migration of hazardous components at their disposal in the environment. The use of complex non-polluted natural objects, such as soil, as a control tool for bioassay of sewage sludge, will, in our opinion, give a more objective assessment compared to the use of unpolluted environment.

Certainly, when using sewage sludge as a fertilizer, their toxic properties can be neutralized in soil, but the initial assessment of such a complex object using biotesting methods is important in order to avoid unpredictable consequences.

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TOXICITY TEST OF INDIVIDUAL AND COMBINED TOXIC EFFECTS OF GLYPHOSATE HERBICIDE AND HEAVY METALS ON CHICKEN EMBRYOS

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ABSTRACT

The aim of this study was to determine the individual and combined toxicity of Glialka Star herbicide (glyphosate 360 g/l) and heavy metals (copper and cadmium) on the development of chicken embryos. On the first day of incubation, chicken eggs were injected by 0.1 ml of the test materials. The applied concentration of copper and cadmium sulphate was 0.01% and that of herbicide Glialka Star was 2%. The chicken embryos were examined on day 19 by the followings: rate of embryo mortality, body mass, type of developmental anomalies by macroscopic examination. The body weight was evaluated statistically by one-way ANOVA combined with Dunnett post-test, the embryo mortality and the developmental anomalies were analysed by Fisher test. Our teratogenicity study revealed that the combined administration of heavy metals (copper, cadmium) and glyphosate (K-salt) containing herbicide formulation caused significant reduction in the body weight of embryos and a significant increase in the rate of embryonic mortality and the incidence of developmental anomalies. The joint toxic effect of heavy metals and Glialka Star is an additive effect compared to the individual toxicity of the test materials.

Keywords: *glyphosate, heavy metals, interaction, embryotoxicity, chicken embryo.*

INTRODUCTION

Because the land used for agricultural production is the food source, shelter and breeding place of our wild birds, the pesticides sprayed during the plant protection activities might have influence not only on mature birds but also on the embryos within the eggs. In the environment which is contaminated by pesticides, these materials change the chemical environment of animals and make poisonings possible. The harmful effects of pesticides can appear in acute damage, or destruction of living creatures at a lower or higher level. The destruction of the mature animals can cause the death of the offspring which remain without food and care, even if they are not poisoned. Those who survive the acute poisoning with a decreased resistance can become the victims of different environmental pollution (Várnagy and Budai, 1995).

Recently, the examination of the combination of heavy metals and other chemicals has gained significant ground in both avian (Fejes *et al.*, 2001; Kertész, 2001) and mammalian (Institóris *et al.*, 2001; Pecze *et al.*, 2001) toxicology research studies.

Teratological tests carried out on avian embryos provide useful data for environmental protection and facilitate the development of environmentally friendly chemical plant protection techniques (Várnagy *et al.*, 1996).

The objective of this study was to determine the individual and combined embryotoxic effects of heavy metals (copper, cadmium) modelling the heavy metal load of the environment and an optionally selected pesticide widely applied in the practice (Glialka Star). As the ecotoxicological testing methods used in the practice are mainly limited to study the toxic effect of compounds used alone, data on interactions between pesticides can be regarded as gap-filling information especially in relation to the avian organism.

MATERIALS AND METHODS

For modelling the environmental copper and cadmium load, 0.01% copper sulphate solution (Reanal-Ker Ltd., Hungary) and 0.01% cadmium sulphate solution (Reanal-Ker Ltd., Hungary) was used in individual and joint treatments.

At present, copper is used primarily for wire manufacturing, as a chemical catalyst and for the production of alloys. It is applied as a nutrient in plant cultivation and as a bactericidal, fungicidal and algicidal agent in chemical plant protection. In veterinary medicine copper is used as a feed additive, growth promoter and disease-preventing substance. Cadmium is used in the industry for the production of alloys and for corrosion protection of iron objects. It is also used in the manufacture of batteries, rubber and paint, as well as to stabilize plastics. However, it is also not unknown in the field of chemical plant protection that cadmium-containing compounds have previously been used as fungicides on golf courses (Adriano, 1986).

The herbicide Glialka Star (360 g/l glyphosate [K-salt], Monsanto Hungária Ltd., Budapest, Hungary) was used in individual and joint treatments in typical field application rate (2%). It is a phosphorous-containing pesticide with 360 g/l glyphosate K-salt as active ingredient and assigned to marketing category III. It is used widely on arable land as well as in horticulture and viticulture, for drying and killing annual and perennial single and dicotyledonous plants. The product is not toxic to bees and to fish (NFCSO, 2011). The studies were conducted with purebred fertile Farm hen's eggs derived from the stock farm of Goldavis Ltd. (Sármellék, Hungary). The eggs were incubated in a Ragus type hatcher (Vienna, Austria). During the incubation, the appropriate temperature (37–38°C), air humidity (65–75%) and the daily rotation of eggs were provided (Bogenfürst, 2004). The treatment of eggs (n=45/group) was performed on the day of initiation of hatching. In the individual treatments, solutions and emulsion made from test chemicals in 0.1–0.1 ml end volume were used while in the joint treatments, 0.2 ml of the chemical agents were injected into the air chambers of eggs in each combination (Clegg, 1964; Lutz, 1974). For the preparation of solutions and emulsion as well as in the control treatments, distilled water was used. The incubation was started immediately after the treatments. The processing was conducted two days before the expected hatching on the 19th day of incubation. Within the pathological studies, the body weight of embryos, the number of dead embryos and the macroscopic malformations were determined and recorded. In the case of the body weight data of live embryos, statistical comparisons among the groups were made with one-way analysis of variances (ANOVA). Because the results showed significant differences, Dunnett tests were also performed. In the case of the biometric processing of the embryonic mortality and malformations, exact test according to Fisher was used.

RESULTS AND DISCUSSION

The embryonic mortality in the control group treated with distilled water was 4.65% (Table 1). The rate of embryonic mortality could be considered sporadic which made it possible to use that group as a frame of reference. There was no any malformation in this group (Table 2). On the effect of the injected distilled water in the control group, the average body weight of the embryos was 23.24±1.85 g (Table 3).

On the effect of the 0.01% copper sulphate solution the embryonic mortality significantly ($p<0.05$) increased to 20.45% in comparison to the control group (Table 1). Malformations occurred two times in that group (5.71%). Types of developmental abnormalities were the followings: open chest cavity, retarded development, and malformation of feet (Table 2). Injection of copper sulphate decreased the body weight of embryos (22.56±2.06 g) in comparison to the control group (23.24±1.85 g), but the difference could not be proved statistically (Table 3).

When the cadmium sulphate was used individually, almost one half of the treated animals died (47.73%; $p<0.001$) that was statistically significant compared to the control group (Table 1). There was not any embryo showing developmental anomalies in the group (Table 2). The individual administration of cadmium sulphate in 0.01% concentration on the day 0 of the incubation caused a significant ($p<0.05$) reduction in the body weight of embryos (21.86±2.25 g) compared to the control data (Table 3).

The rate of embryonic mortality significantly ($p<0.01$) increased (30.95%) in the group treated with herbicide (Glialka Star) individually (Table 1). When the glyphosate-containing herbicide was used the incidence of developmental anomalies was sporadic (3.44%) (Table 2). The developmental anomalies appeared as malformation of feet, open body cavity and retarded growth. After the single administration of the herbicide formulation in 2% concentration on the day 0 of the incubation, significant decrease ($p<0.01$) was established in the embryonic body weight (21.84±1.70 g) in comparison with the values of the control group (23.24±1.85 g), (Table 3).

As a result of the combined application of copper sulphate and the herbicide, the rate of embryonic mortality reached 53.48% that was statistically significant compared to the control group ($p<0.001$) (Table 1). Three of the living embryos (3/20) as a result of combined administration showed developmental anomaly. The incidence of developmental anomalies was significantly ($p<0.05$) higher than the control. Looking at the types of development disorders the most frequent problem was retarded development, malformation of feet, lack of right eye, bulging of left eye, the shortening of the beak mandible, and open chest cavity (Table 2). The combined administration of copper sulphate and glyphosate containing herbicide formulation resulted in a significant decrease in the average body weight (21.05±1.73 g) of embryos compared to both the control group ($p<0.001$; 23.24±1.85 g) and the group treated with either copper sulphate ($p<0.01$; 22.56±2.06 g) alone (Table 3).
In comparison with the values of the control group, the rate of dead embryos increased significantly ($p<0.001$) in the group treated with cadmium sulphate and glyphosate-containing herbicide product (Glialka Star). The rate of embryonic mortality was 83.33% (Table 1). In the group the occurrence of development disorders (42.85%) was at a significant higher level ($p<0.01$) than in the case of individual treatments (cadmium sulphate: $p<0.01$; Glialka Star: $p<0.05$). The developmental anomalies could be identified as hernia of brain, retarded development, malformation of feet, open body cavity, bulging of eye (Table 2). The combined treatment with cadmium sulphate and Glialka Star induced significant decrease of the embryonic body mass (19.13±1.35 g; $p<0.001$) compared with the control and individually treated groups ($p<0.01$) (Table 3). So, it can be established that the combined treatment resulted in an increased embryotoxic effect in comparison with the individual embryo damaging effect of the used compounds separately.
In former teratogenicity tests (Fejes, 2005) performed on chicken embryos using a series of concentrations of copper and cadmium sulphate solution (1.0%, 0.1%, 0.01%, 0.001%) the rate of embryonic mortality and developmental anomalies proved to be significantly ($p<0.05$) increased in comparison to the control groups.
In teratogenicity studies of various copper salts conducted on pregnant hamsters it was found that the high doses of copper sulphate had increased the rate of embryonic mortality, malformations in uteri and foetal deformities. Malformation of the heart appeared to be a of the tested copper compounds (Ferm and Hanlon, 1974).
Similarly, to previous results (Juhász *et al.*, 2006; Szabó *et al.*, 2011), according to the pathological studies it was established that individual treatments with cadmium sulphate significantly ($p<0.05$) increased the embryonic mortality.
Based on the results of our teratogenicity studies on single administration of injected copper sulphate, it can be established that at 0.01 concentration level has a slight embryotoxic effect. The teratogenic effect was not justified. On the base of our teratogenicity studies on single administration of injected glyphosate containing herbicide formulation (Glialka Star) caused a significant reduction in

the body weight of embryos and markedly increased the rate of embryonic mortality. The herbicide Fozát 480 (glyphosate) in individual and combined administrations significantly increased the embryonic mortality (Szabó *et al.*, 2017).

Table 1. The number and rate of dead embryos on day 19 of incubation

Treated groups	Number of dead embryos/ number of fertile eggs (pcs)	Rate of dead embryos (%)
Control	2/43	4.65
Copper sulphate	9/44[a1]	20.45
Cadmium sulphate	21/44[a3]	47.73
Glialka Star	13/42[a2]	30.95
Copper sulphate + Glialka Star	23/43[a3, b]	53.48
Cadmium sulphate + Glialka Star	35/42[a3, c, d]	83.33

[a]Significant difference as compared to the control group ([a1]$p<0.05$; [a2]$p<0.01$; [a3]$p<0.001$).
[b]Significant difference as compared to the group treated with copper sulphate alone ([b]$p<0.05$).
[c]Significant difference as compared to the group treated with cadmium sulphate alone ([c]$p<0.05$).
[d]Significant difference as compared to the group treated with Glialka Star alone ([d]$p<0.01$).

Table 2. Number and rate of malformed embryos on day 19 of incubation

Treated groups	Number of malformed embryos/ number of alive embryos (pcs)	Rate of malformed embryos (%)
Control	0/41	0.00
Copper sulphate	2/35	5.71
Cadmium sulphate	0/23	0.00
Glialka Star	1/29	3.44
Copper sulphate + Glialka Star	3/20[a1]	15.00
Cadmium sulphate + Glialka Star	3/7[a2, b, c]	42.85

[a]Significant difference as compared to the control group ([a1]$p<0.05$; [a2]$p<0.01$).
[b]Significant difference as compared to the group treated with cadmium sulphate alone ([b]$p<0.01$).
[c]Significant difference as compared to the group treated with Glialka Star alone ([c]$p<0.05$).

Table 3. Embryonic body weights (g) on day 19 of incubation

	Control	Copper sulphate	Cadmium sulphate	Glialka Star	Copper sulphate + Glialka Star	Cadmium sulphate + Glialka Star
Number of embryos (n)	41	35	23	29	20	7
Average (g)	23.24 ±1.85	22.56 ±2.06	21.86 ±2.25[a1]	21.84 ±1.70[a2]	21.05 ±1.73[a3, b]	19.23 ±1.35[a3, c, d]

[a]Significant difference as compared to the control group ([a1]$p<0.05$; [a2]$p<0.01$; [a3]$p<0.001$).
[b]Significant difference as compared to the group treated with copper sulphate alone ([b]$p<0.01$).
[c]Significant difference as compared to the group treated with cadmium sulphate alone ([c]$p<0.01$).
[d]Significant difference as compared to the group treated with Glialka Star alone ([d]$p<0.01$).

CONCLUSION

Our teratogenicity study revealed that the combined administration of injected copper and cadmium sulphate as well as glyphosate-containing herbicide product (Glialka Star) caused a significant reduction in the body weight of the embryos and markedly increased the rate of embryonic mortality. The combined toxic effect of copper sulphate and glyphosate, and cadmium sulphate and glyphosate induced additive effect compared to the individual toxicity of the test materials. The test conducted on day 19 of incubation showed a significant increase in embryonic mortality when heavy metals and herbicide were applied individually. The occurrence of developmental abnormities in the groups receiving individual treatments remained at a relatively low level (3.44-5.71%). However, due to the combined treatment the rate of malformed embryos was highly increased (15.00-42.85%) resulted in synergistic effect. The rate of mortality due to the combined administration was definitely increased over 50% compared to the individual toxicity of heavy metals and the herbicide. In the groups treated with both cadmium sulphate and Glialka Star the body weight of embryos showed a significant decrease compared to both the control and individually treated groups.
Beside the injection treatment method applied during the studies it would be advisable to perform complete examinations with spraying (or bathing, immersing) treatments that can be served as a model of expositional circumstances during the plant protection practice and compare the results achieved from different treatment technologies.

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Review paper

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FOSTERING GREEN ECONOMY THROUGH NEW FINANCIAL INSTRUMENTS IN CENTRAL BANKS' PORTFOLIOS

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ABSTRACT

The green economy implies the use of renewable energy from biomass, solar panels, wind power plants and recycled waste, while providing the environmental protection, human well-being and sustainable economic progress. As with other investments, the important question is how to find sufficient sources of funds and get investors interested in these projects. Global green bond issuance started along with the increasing need for financing green businesses and technologies, resolving climate change issues and financing through efficient emission of green securities and trading on markets. The proceeds of these bonds are explicitly used to finance new or existing green projects. This paper examines the concept and evolving of green bonds with emphasis on the new role of central banks in greening financial systems and its impact towards a green economy. The green bond market dates back to 2007, with launching the World Bank Green Bonds programme in 2007 and the Climate Awareness Bonds by the European Investment Bank (EIB) in 2008 for financing renewable energy and energy efficiency projects. The significance of making bond between sustainable economic development and environmental issues is evident through the rapid growth of these green instruments. The objective of this research was to identify new types of financial instruments intended to improve ecological projects, as well as to compare effects of green bonds utilization in different countries and by different institutions. The results show the improvement of the financial market and investor profits, while there is simultaneously significant growth of green projects pointing to the benefits of using this new form of financial instrument in promoting the green economy.

Keywords: *green economy, sustainable and responsible investment, central banks, green bonds.*

INTRODUCTION

The green economy concept is based on solid foundations of environmental protection, social development and economic progress. The idea of "green economy" is perceived differently by each country depending on a number of factors. Recently there has been a sharp increase in global interest in greening and the reasons lie in the fact that people slowly but surely raise their environmental awareness. With the process of globalization the enormous scale of the ecological crisis, the disturbance of ecosystems, and the overexploitation of natural resources also arise.

The term green economy was first used in 1989 in *Blueprint for a green economy*, composed by a group of leading British economists (Pearce et al., 1989). Green bonds concept is relatively new trend representing support to environmentally responsible finance and investment practices. The bond between sustainable economic development and environmental issues is the essence of this principle. UNEP (United Nations Environment Program) defines the green economy as an economy that results in improved human well-being and social equality, with a significant reduction in environmental risks and further environmental degradation.

The utilization of the principles of the greening should reduce the harmful effects of global climate change while ensuring development economic strategies. The green economy implies the use of renewable energy from biomass, solar panels, wind power plants and recycled waste, while providing the environmental protection, human well-being and sustainable economic progress. As with other investments, the important question is how to find sufficient sources of funds and get investors interested in these projects. For investors trying to achieve both sustainability and financial objectives, green bonds have emerged as a significant tool toward the UN Sustainable Development Goals (SDGs). Green bonds are fixed income securities whose proceeds are used to finance new or existing eligible green projects (BIS, 2019).

The main actors driving green finance development are central banks, international financial institutions, financial regulators, institutional investors and commercial banks. Central banks have now taken a leading role within the financial system using a wide range of financial products to support the greening of the financial system. Through the literature, the main arguments justifying new central bank roles are the environmental and sustainability challenges including the financial and macroeconomic risk argument, the market failure argument, and an argument relating to the role of central banks as credible and powerful actors, especially in developing countries (Volz, 2017).

The objective of this paper is to explore the effect of inclusion of green bonds in the central banks portfolio considering types of green bonds, risk and return for investors.

MATERIAL AND METHODS

Empirical analysis comprises central banks in their portfolio expansion with new financial instrument, green bonds. The first part of the analysis looks at changing role of central banks and their adjustment in incorporating environmental sustainability objectives into their portfolios. In this paper it is explored how sustainability considerations can be integrated into the central banks framework. The second part presents the findings of the green bond structures by issuer type profiles and regions. The benefits and the risks were also observed through

generating diversification as well as significance of features on different bond types for issuers and investors.
In a time range of 5 years, the rapid issuance and growing demand shows significant rising from less than $50 billion in 2014 to close to $260 billion in 2019 (Climate Bonds Initiative, 2020). The paper reviews features and trends in green bonds through analysing data from the official websites of the observed central banks and financial institutions, as well as survey reports which follow the growth trends and movements of these bonds.

RESULTS AND DISCUSSION

A significant group of authors point out that the green economy is experiencing a real expansion after the last financial crisis (Georgeson et al. 2017; Davies 2013; Bowen et al. 2009). The need for new forms of investment and innovative financial resources were the impetus for further growth and development of this economic form. It is generally accepted that the concept of a green economy should improve human well-being and reduce inequality, while reducing harmful human impacts on the environment (Newton and Cantarello, 2014). According to these authors, it enables: reduction of atmospheric warming by reducing greenhouse gas emissions, stabilization of average air temperature, adequate management of natural resources, increasing the country's resilience to environmental changes or natural disasters and catastrophes. In addition to these environmental benefits of the green economy, it also brings social and economic benefits. In terms of economic effects, it is primarily referred to increasing economic growth and employment rates.
Considering the role of central banks in enhancing green finance, it includes possibility that financial firms may face climate or environment-related liability risks that could arise if agents suffering losses related to climate change or environmental damages seek compensation from those they hold responsible for their damage, including carbon extractors or emitters and environmental polluters more generally. To the extent that central banks are tasked with guarding financial stability, these risks need to be considered in central banks’ financial stability and macroprudential policy frameworks (Carney, 2015). Green bonds offer similar yields, ratings and return profiles to other fixed income investments, and they fund projects that are making a tangible and measurable impact in the effort to address environmental, social and economic challenges. In other words, they offer additional impact benefits for investors, without additional financial risk (Graff and Hauter, 2017). Unlike classic bonds, green bonds support the financing of projects in the field of climate change mitigation. Their value lies in the fact that the issuer undertakes directly collected funds to the financing of projects that have a positive impact on the environment. These funds are raised exclusively to finance or refinance "green projects" and they are focused on energy efficiency (including efficient buildings), sustainable waste management, sustainable land use (including sustainable forestry and agriculture), conservation of biodiversity, clean transport, sustainable water management (including clean and/or drinking water) and adaptation to climate change (Vella, 2018). Green bonds are fixed income

securities whose proceeds are used to finance new or existing eligible green projects, projects to combat pollution, climate change or the depletion of biodiversity and natural resources (Ehlers and Packer, 2017). Green bonds were created to fund projects that have positive environmental and/or climate benefits. In Table 1. the main types of green bonds are presented and we can notice that majority of the green bonds issued are green “use of proceeds” or asset-linked bonds. There have also been green "use of proceeds" revenue bonds, green project bonds and green securitised bonds. Proceeds from these bonds are *earmarked* for green projects but are backed by the issuer’s entire balance sheet (Climate Bond Initiative, 2020).

Table 1. Types of green bonds

Type	***Proceeds raised by bond sale are***	***Debt recourse***	***Example***
"Use of Proceeds" Bond	Earmarked for green projects	Recourse to the issuer: same credit rating applies as issuer's other bonds	EIB "Climate Awareness Bond" (backed by EIB); Barclays Green Bond
"Use of Proceeds" Revenue Bond	Earmarked for or refinances green projects	Revenue streams from the issuers though fees, taxes etc. are collateral for the debt	Hawaii State (backed by fee on electricity bills of the state utilities)
Project Bond	Ring-fenced for the specific underlying green project(s)	Recourse is only to the project's assets and balance sheet	Invenergy Wind Farm (backed by Invenergy Campo Palomas wind f.)
Securitisation (ABS) Bond	Refinance portfolios of green projects or proceeds are earmarked for green projects	Recourse is to a group of projects that have been grouped together (e.g. solar leases or green mortgages)	Tesla Energy (backed by residential solar leases); Obvion (backed by green mortgages)
Covered Bond	Earmarked for eligible projects included in the covered	Recourse to the issuer and, if the issuer is unable to repay the bond, to the	Berlin Hyp green Pfandbrief; Sparebank 1 Bolligkredit green covered bond

	pool	covered pool	
Loan	Earmarked for eligible projects or secured on eligible assets	Full recourse to the borrower(s) in the case of unsecured loans. Recourse to the collateral in the case of secured loans, but may also feature limited recourse to the borrower(s)	MEP Werke, Ivanhoe Cambridge and Natixis Assurances (DUO), OVG
Other debt instruments	Earmarked for eligible projects		Convertible Bonds or Notes, Schuldschein, Commercial Paper

Source: Adapted by the author, from The Climate Bonds Initiative (2020)

There are several main reasons for introducing green bonds to the market and into the central bank portfolio. We can observe it through the mutual benefit of investors, the state, the financial market and other participants in that market. From the investor's point of view, the modern era should involve different factors and partners into their investment processes, so that investors benefits could be: funding green projects without taking any additional risk or cost, greater transparency into a bond's use of proceeds, being in line with the basic principles on which sustainable development is based on along with environmental protection, reporting on climate impact of fixed income investments to their end asset owner. For years, investors have been growing their understanding of how environmental, social and governance issues in the real world affect their portfolios. Now understanding is also growing of how investors' actions also have outcomes in the real world - along with expectations of what investors should be doing about those outcomes (PRI, 2020). Green bonds have some additional transaction cost because issuers must track, monitor and report on use of proceeds. However, many issuers, especially repeat green bond issuers, offset this initial cost with the following benefits: highlights their green assets/business, positive marketing story, diversify their investor base and joins up internal teams in order to do the investor roadshow (environmental team with Investor relations and other business). The World Bank provides data on the estimated impact of its portfolio, e.g. two energy saving projects in China expect to save 12.6 million tons of CO_2 equivalent annually through US$400 million of financing from green bonds.

According to the current data of the Climate Bond Initiative (CBI), global green bond and green loan issuance reached USD 257.7 bn in 2019, making a new global record. This presents huge increase in demand for this form of financial innovation since it started, thus justifying its creation. The green bond market dates back to 2007, when institutions such as the European Investment Bank and the World Bank issued bonds for financing, renewable energy and energy efficiency projects. Since its introduction to the present day, the green bond market has diversified, with new issuers worldwide, especially in developed countries. Starting in 2007 by multilateral development banks, the market is mainly driven by issuers from the public sector, municipalities and states, government agencies, national and international banks.

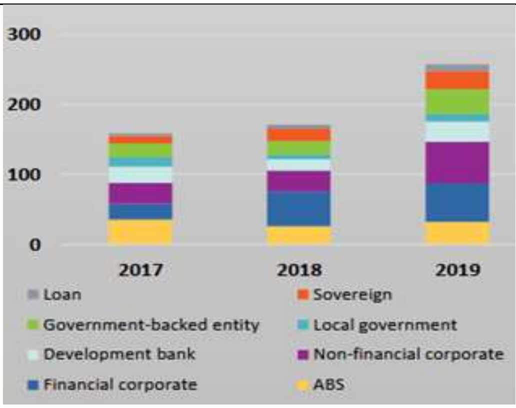
Annual Green bond issuance 2017 – 2019 billions of US dollars	Regional issuer type split in 2019
	

Figure 1. Annual issuance of green bonds by issuers and issuer split in 2019
*Source: Climate Bonds Initiative (2020)

The data shows that there is a continuing rise in Sustainability/SDG bond frameworks in 2019, looking between green and social eligibility criteria, which allow the issuer to classify a bond as “green”, “sustainability” or “social” depending on the use of proceeds. This framework allows the issuer to make a clear distinction between bonds that finance environmental and social projects, if relevant. This makes it easier for investors with a dedicated mandate to identify bonds that comply with their investment criteria. When observing regionally, USA, China and France are top-ranked countries. Together they accounted for 44% of global issuance in 2019.

Regional analysis of issuer types compared to 2019 shows the following differences: We can notice the structural differences in the types of issuers that access debt markets in each region. In the USA, significant issuance volume from Fannie Mae leads to a very high share of ABS deals and Muni bonds (issued by US local governments) are also common. In Europe, the share of government-backed entities is much higher than in most other regions owing to the historically greater role played by European states in economic activities and planning. In certain countries, for instance Brazil, local governments have limited abilities to issue debt, since it requires a sovereign guarantee. Comparing the profiles in 2019 with those up to 2018, it is also clear that less developed regions - namely Africa and Latin America and the Caribbean (LAC) - tend to have a more “volatile” mix of issuer types than their more developed counterparts. Sovereign issuance in Europe also picked up in the last two years (especially in 2018). Overall, the issuer type mix in Europe is consistently the most varied and balanced of the regions. Asia-Pacific region saw a changing mix with an increase in non-financial corporate issuance in 2019, coupled with a decrease in financial corporate and development bank activity. In North America, the corporates - both financial and non-financial are growing in share while local governments are dropping on a relative basis.

CONCLUSION

The growth of green bond markets fosters the possibility to finance the implementation of green economy projects and to achieve the Sustainable Development Goals. This means more low-carbon, energy- and resource-efficient circular projects. Central banks are now playing an active and significant role in promoting the green and sustainable global economy. Integrating sustainability deliberations should help in diminishing the impact of natural disasters, simultaneously considering environmental and social sustainability issues that can affect the economy and financial markets.

Central banks can use various tools to support the greening of the financial system as it is including green bonds in their portfolios. The practice shows multiple benefits for investors, governments, social as well as environmental benefits. Green bonds can stimulate financial markets, regulatory and economic policy reforms to boost green growth and to help countries significantly accelerate green economy transformation.

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Original Scientific paper

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PRE-TREATMENT AND INVESTIGATION OF WHEAT STRAW AND HEMP SHIVES FOR BINDER-LESS FIBREBOARD PRODUCTION

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ABSTRACT

Wood-based panels (WBP) comprise a considerable part in the output of the European wood industry, GDP, and export. Over 30% of fibreboards and about 50% of other boards used in constructions and carpentry are produced in Europe. Industrially produced WBP contain synthetic adhesives determining mechanical properties and being made from oil are often toxic present health risks. Synthetic adhesives may comprise up to 15 % of the total WBP mass and up to 60 % of the total production costs. Production costs of WBP are important under the circumstances of rising oil prices. Moreover, synthetic bonding agents rather often is a source of emissions of formaldehyde and another problems with advancement of WBP solutions being offered by synthetic adhesives without formaldehyde (polymeric methylene diphenyl di-isocyanate – PMDI), or by natural adhesives from renewables (e.g., tannins or soy flour), or by pre-treatments activating bonding agents contained in the source material. The availability of raw materials for WBP is still another problem under the circumstances of the rate of population growth exceeding the rate of the regeneration of wood resources. Expanding the diversity of raw materials for production of WBP by utilization of agricultural residues containing components like wood is one of the possible solutions. The present study is aimed at development of technology of binder-less fibreboards made of steam-exploded agricultural residues, such as wheat straw (*Triticum aestivum* L.) and industrial hemp (*Cannabis sativa* L.) shives. Some aspects of the study, like differences in chemical composition, thermal properties of raw and pre-treated materials potentially affecting binder-less fibreboard bonding, presented.

Keywords: *Wheat straw, Hemp shives, Steam explosion pre-treatment, Binder-less fibreboard.*

INTRODUCTION

The concept of bio-refinery (Gravitis and Suzuki, 1999) using agricultural residues for value added products meet the European 2020 Strategy where a bio-economy is the key element for innovative product lines based on renewable biological resources, including waste streams or production residues (European Comission, 2012). Industrial hemp (*Cannabis sativa* L.) species approved for cultivation and mainly grown for the long bast fibres widely used in many composites (Shahzad, 2012) becoming the leading crop in Europe, including Latvia (Raymunt, 2020). The hemp woody inner part, called shives, make up to 75% of the stalk mass and have an excellent potential to be used as raw material for value added bio-composites because of its chemical components being similar to wood (Paze *et al.*, 2015). However, almost 2/3 of industrial hemp shives in Europe are used for animal bedding and the rest 1/3 for construction and garden mulch (Romanese, 2019). Some preliminary studies of complex processing of industrial hemp shives have been awarded to products of high added value, such as furfural and self-binding panels (Brazdausks *et al.*, 2015) with acoustic application (Brencis *et al.*, 2015). A significant amount of annual agricultural crops in Latvia takes different cereals from which wheat (*Triticum aestivum* L.) cover about 2/3. After the grain harvesting the straw is the by-product with a limited application. Some surveys demonstrate high potential of wheat straw obtaining fibreboards (Halvarsson, Edlund and Norgren, 2010; Sitz *et al.*, 2017), fuel pellets (Adapa, Tabil and Schoenau, 2011), and bioethanol (Fang, Deng and Zhang, 2011). Also, the wheat straw enhance digestibility in ruminants (Viola *et al.*, 2008).

Steam explosion (SE) is one of efficient pre-treatment technologies subjecting the raw material to hydrothermal modification. In the procedure of auto-hydrolysis the main chemical components of raw material are restructured: the main part of hemicelluloses is destructed, the cellulose is partly depolymerised and a part of lignin – depolymerised and deposited on cellulose fibres separated by decompression (Negro *et al.*, 2003). During the hot-pressing of the pre-treated substance the modified lignin and destruction products of the hemicelluloses and extracts act as bonding agents forming new composite of rather good mechanical properties. SE is a competitive technology for processing diverse raw materials to provide added value of biomass (Zimbardi *et al.*, 2002; Quintana et al. 2009).

Wood-based panels (WBP) are value added composites made of wood particles usually bonded by formaldehyde-containing resins for many construction applications. However, these products emit free formaldehyde, which is classified as carcinogenic (IARC, 2006). Availability of raw materials for WBP is still another problem under the circumstances of the rate of population growth exceeding the rate of the regeneration of wood resources. Expanding diversity of raw materials for production of WBP by utilisation of agricultural residues containing components like wood is one of possible solutions.

The present study demonstrates the potential of wheat straw and hemp shives for binder-less fibreboard production by using the pre-treatment of SE.

MATERIALS AND METHODS

The most locally cultivated and available retted industrial hemp species of Futura 75 (Kraslava district), Finola and Uso 31 (Jelgava district) were used as hemp shives raw materials. The percentage content of fibre and shives for Futura was 33:62, for Finola and Uso 15:80, respectively. Only hemp shives with a maximum

of 2% of residual fibre content were used for further investigation. Wheat straw was delivered from a farmer in Limbaži district. All raw materials were crushed in a knife mill through a sieve with openings of Ø10 mm removing the fraction of ≤ 0.5 mm because of high ash content (up to 55% in the case of Futura 75).
The crushed raw materials were pre-treated in a steam explosion (SE) device with 0.5 l batch reactor at a temperature range of 200–240°C. The severity factor (R_0) combining temperature, *T*, and reaction time, *t* ($R_0 = t*exp[(T-100)/14.75]$) was chosen to express the SE process. The selected experimental design of SE process is shown in Table 1.

Table 1. Experimental design of raw materials for SE process.

Sample	T, ^{0}C	p, bar	t, min	$\log R_0$
SE200/1	200	15	1	2.94
SE200/3	200	15	3	3.42
SE220/2	220	23	2	3.83
SE240/1	240	33	1	4.12
SE240/3	240	33	3	4.60

The raw and pre-treated materials were characterized by detected chemical components (NREL/TP-510-42618 and NREL-TP-510-42622) and thermal analysis in terms of differential scanning calorimetry (DSC, heating rate 10°C min^{-1} up to 500°C) and thermogravimetry (TG, heating rate 10°C min^{-1} up to 800°C).
One layer binder-less boards (6 x 150 x 180 mm) were fabricated from crushed (sieve 2 mm) SE materials (fraction 0.05–2 mm, moisture content 5 ± 0.5%) at a constant temperature of 175°C, under maximum pressure of 4 MPa, and pressing rate of 1 min/mm, at the set density level of 900 kg m^{-3}. The fabricated boards were cut to the bending test specimen dimensions (50 x 150 mm) and visually evaluated.

RESULTS AND DISCUSSION

Results of detected chemical components of neat and steam-exploded samples (% of oven dry mass) are shown in Fig. 1. The ash content of used neat hemp shives varies between 2.1% (Finola) and 3.1% (Futura 75), being lower than for neat wheat straw (4.0%). The ash content remains approximately the same after the SE pre-treatment for all used crops except for Futura 75 which ash content significantly increases. The glucan content of neat crops, that is the major portion of cellulose, varies between 39.9% (wheat straw) and 43.5% (Finola). The glucan content of all crops significantly decreases after the pre-treatment; However, the glucan content of Finola and wheat straw increases again at the higher severities of the pre-treatment at SE240/1 and SE240/3 due to the destruction of hemicelluloses. The detected hemicelluloses content (xylan, galactan, arabinan, mannan and acetyl groups) of neat crops varies between 23.2% (Finola) and 26.7% (Uso 31) and rapidly decreases with the lowest severity of the pre-treatment (SE200/1) from 22.5% (wheat straw) to 17.0% (Futura 75) down to 1.8% (Futura 75) – 0.7%

(Finola) at the highest severity of the pre-treatment (SE240/3). The detected lignin content of neat crops varies between 19.4% (wheat straw) and 26.6% (Uso 31) and significantly increases up to 44.0% (Futura 75) – 47.4% (Uso 31) at the highest severity of the pre-treatment.

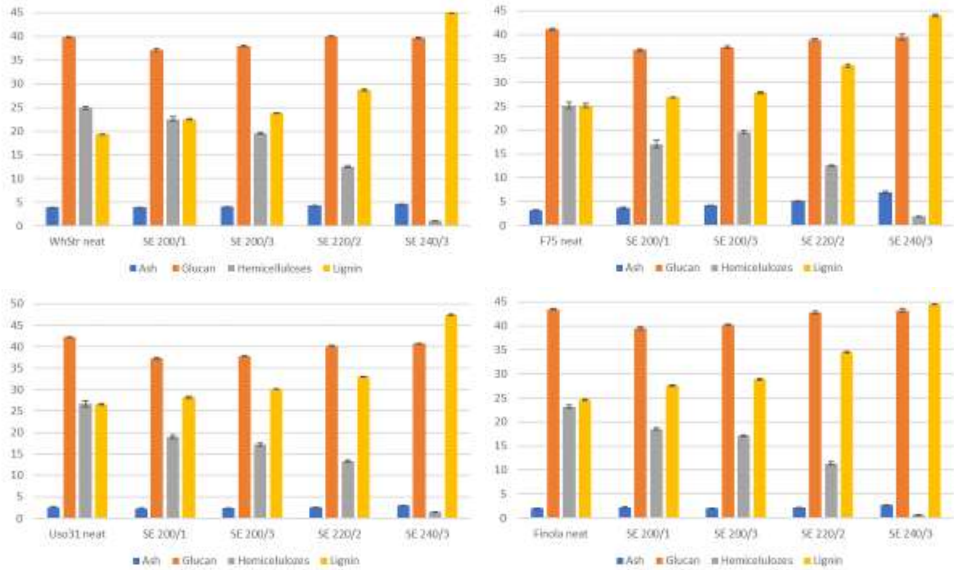

Fig. 1. Chemical components of neat and pre-treated crops (% of oven dry mass).

DSC analysis of all detected raw materials indicated three main exotherms which maximums are at the temperature ranges of 295–315°C, 345–390°C and 420–425°C, and refer to hemicelluloses, cellulose and lignin, respectively (Brys *et al.*, 2016). DSC analysis of pre-treated materials revealed that exotherms maximums at 300–318°C, referring to the degradation of hemicelluloses, remains only for the samples pre-treated at 200°C for 1 min. The exotherms referred to cellulose at 333–390°C and lignin at 390–424°C of all pre-treated samples remains at the same range independent on the pre-treatment conditions (Fig. 2 left).
DSC analysis revealed that softening of Futura 75, Finola, Uso 31, and wheat straw pre-treated at 200°C, 1 min occurs in the temperature ranges of 165–220°C, 180–230°C, 170–215°C, and 170–190°C, respectively. The softening of Futura 75, Finola, Uso 31, and wheat straw pre-treated at 220°C, 2 min occurs in the temperature ranges of 137–140°C, 135–138°C, 163–192°C, and 135–137°C, respectively (Fig. 2 left). The softening of Futura 75, Finola, Uso 31, and wheat straw pre-treated at 240°C, 3 min occurs in the temperature ranges of 130–160°C, 132–140°C, 125–135°C, and 130–150°C, respectively.
Maximum mass losses of all detected raw materials indicated between 336°C and 367°C with sample residue of 15–23% at the end of the process (at 500°C). Maximum mass losses of all detected steam-exploded materials indicated between 302 and 368°C with sample residue of 3–19% at the end of the process (Fig. 2 right).

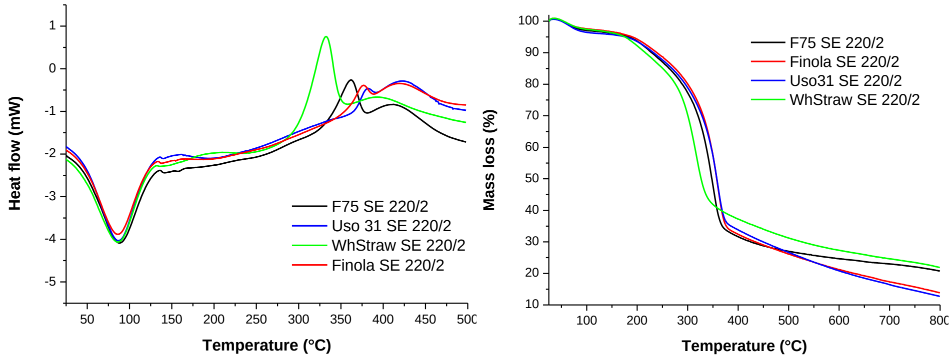

Fig. 2. DSC (left) and TG (right) curves of the crops pre-treated at 220°C, 2 min.

The quality of the boards obtained from the crops pre-treated at 200°C, 1 min and 3 min is poor, because of the particles were not able to be glued strongly and the edges of the boards were not straight after the cutting due to brittleness (Fig. 3, left column). The worst quality of the boards obtained from the samples pre-treated at 200°C demonstrate wheat straw. The boards obtained from the samples pre-treated at 220°C for 2 min and 240 for 1 min have the best quality meeting the minimal technological requirements during the preparation and fabrication of the board (Fig. 3, middle column). The quality of the boards obtained from the samples pre-treated at 240°C for 3 min is quite good (Fig. 3, right column), however, emissions of irritating eyes volatiles were observed during the hot-pressing of the boards.

Fig. 3. Binder-less boards of pre-treated (from above to bottom) hemp shives of Futura 75, Finola, Uso 31, and wheat straw at the 200, 220, and 240°C (from left to right).

CONCLUSIONS

Steam explosion pre-treatment at 200°C, 1 min resulted to significant decrease in cellulose content for all crops (36.8%–39.5%), however, the increase of pre-treatment temperature up to 240°C resulted to the increase of cellulose of Finola and wheat straw up to the level of neat crop (43.2% and 39.7%, respectively). Hemicelluloses content of all pre-treated crops rapidly decreases from 22.5% (wheat straw) – 17.0% (Futura 75) at the severity of 200°C, 1 min down to 1.8% (Futura 75) – 0.7% (Finola) at the highest severity of 240°C, 3 min. The detected lignin content of neat crops varies between 19.4% (wheat straw) and 26.6% (Uso 31) and significantly increases up to 44.0% (Futura 75) – 47.4% (Uso 31) at the highest severity of the pre-treatment.

The softening feature, suggesting the material pressing temperature, occurs at temperature range of 165-180°C for the crops pre-treated at 200°C, 1 min; 135-163°C for the crops pre-treated at 220°C, 2 min; and 125-132°C for the crops pre-treated at 240°C, 3 min.

The boards obtained from the samples pre-treated at 220°C for 2 min and 240 for 1 min have the best quality meeting the minimal technological requirements during the preparation and fabrication of the board which will be used in the further research.

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Original Scientific paper

UDC 632.782(478)

BIOLOGICAL PECULIARITIES OF *CYDALIMA PERSPECTALIS* (WALKER, 1859) IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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ABSTRACT

The box tree moth *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), dangerous pest boxwood plants in East Asia (China, Japan and Korea), is currently officially present in 35 countries of the Europe. In the plantations of *Buxus sempervirens* in the central zone of the Republic of Moldova, a new invasive pest *C. perspectalis* was revealed in several localities. As a result of studying the biological peculiarities, it was found that the phytophage develops in the republic in three generations, wintering at the stage of larvae of 2-3 ages. The flight of imago in spring begins in May, the next generations appear in the middle and end of summer. Flight of imago and oviposition can continue until September-October with overlapping generations. The development of eggs at a temperature of + 25 ± 2 ^{o}C continued 3-5 days on average, the development of larvae was 20-36 days, the stage of the pupa – 12 ± 1.0 days. Lowering the temperature increased the duration of each stage. Moldovan populations box tree moth, as well as all the other populations described earlier from Asia and Europe, have several different types of wing colouring – typical (white), intermediate and melanic morphs. Pest monitoring is required to control population density. To manage the density of pest populations, it is necessary to use both traps with sex pheromone and microbiological and chemical preparations, depending on the situation.

Key words: *Cydalima perspectalis (Walker, 1859), invasive species, biological peculiarities, life cycle, monitoring pest density.*

INTRODUCTION

The natural range of the box tree moth *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) is the humid subtropical regions of East Asia: in China (Walker, 1859), Japan (Inoue, 1982) and Korea (Park, 2008). In addition, Hampson (1896) mentioned its occurrence in the Indian state Himachal Pradesh but this was the only record for India and therefore it is listed as unreliable and currently absent to India (CABI, 2020). Some authors point out that the Russian Far East – Primorsky Krai (part of it) is a native range for *C. perspectalis* (Kirpichnikova, 2005). However, there is an opinion that, apparently, the moth is not native there, because boxwood plants were also introduced into the region at one time (CABI, 2020). In Europe this pest was firstly registered in 2007, in Southern Germany. Imago and larvae *C. perspectalis* were detected when they fed on *Buxus* plants near the port of trans-shipment in Weil am Rhein (Baden-Württemberg) (Billen, 2007). Since the infection was significant and spread over a large area, it was suggested that the invasion occurred several years earlier – probably 2-3 years before its detection (Billen, 2007; Krüger, 2008). The author assumed that the possible pathway of the introduction is the Rhine port in Weil am Rhein and nearby Basel in Switzerland where large shipments of Chinese imports are arriving regularly. At the April of 2020, i.e. thirteen years after the first record in Germany and Switzerland, *C. perspectalis* was officially present in 35 countries of the Europe (Feldtrauer et al., 2009; Mitchell, 2009; Muus et al., 2009; Aistleitner, 2010; Iamandei, 2010; Mally & Nuss, 2010; EPPO, 2011, 2016; Safian & Horvath, 2011; Hizal, 2012; Koren & Črne, 2012; Pastorális et al., 2013; Gninenko et al., 2014; Ostojić et al., 2015; Strachinis et al., 2015; Bury et al., 2017; Bakay & Kollar, 2018; Plant et al., 2019; CABI, 2020). It is known, that imago the box tree moth are good flyers and can fly long distances. But most researchers believe that infested *Buxus* plants are the main pathway and source of infection, because eggs and larvae, especially of younger instars, are closely related to the host plant and are difficult to visually determine (Leuthardt et al. 2010; van der Straten & Muus 2010; John & Schumacher 2013; Nesterenkova et al., 2017). Thus, in Russia, in the Caucasus region *C. perspectalis* also probably brought with plants imported from Italy for landscaping the Olympic village in Sochi (Gninenko et al., 2014). In Europe, the pest was widespread due to the fact that the European Union is a free market for living plants and *C. perspectalis* was not included in the EU Plant Health Directive or was not classified as a quarantine pest by the European and Mediterranean Plant Protection Organization (EPPO, 2016). In the Republic of Moldova, this species was first registered in the private sector in 2015 on *Buxus sempervirens* plants (ANSA…, 2018). At the same time, we also detected the settlement of boxwood ornamental plantations in the central zone of the Republic of Moldova – in Chisinau city and its environs. In some places, the pest density was so high that it led to complete defoliation of *Buxus* plants and even to complete drying out of large sites of boxwood plantations. The exact invasion routes of *C. perspectalis* into the Republic of Moldova have not been identified. However, in all probability, this species was imported from Europe along with planting stock. In order to organize successful qualified control over the population density of a new dangerous pest, it is necessary to carefully study its biological special aspects in the new conditions. The aim of this paper is to describe the biological particularities of box tree moth with prognosis of its future spread and damages in the Republic of Moldova.

MATERIALS AND METHODS

Observations were carried out in the central zone of the Republic of Moldova – mun. Chisinau during 2016-2020. The number of pests *Cydalima perspectalis* on boxwood plants (*Buxus sempervirens*) was recorded using the sequential method with an interval estimation of population density. In this regard, boxwood twigs were periodically cut off – 10 samples per 1 meter of boxwood hedge were taken. Larvae of all instars were brought to pupation under laboratory conditions at the +24±2 ^{o}C, 16-h day length, in plastic and gaze cages (10 x 10 x 10 cm). Fresh of *B. sempervirens* branches, partly immersed in water, were used as food for larvae of box tree moth. The adults were identified according to Mally and Nuss (2010).

RESULTS AND DISCUSSIONS

As a result of the surveys, we found that the pest *Cydalima perspectalis* overwinters in the conditions of the Republic of Moldova mainly at the stage of larvae of about the third instar (Fig. 1 a). The measurements showed that the length of the larvae was on average 0.9 cm and fluctuations from 0.6 to 1.2 cm. The width of the head capsule averaged up to 1 mm. From November to December, we also found a few cocoons with wintering larvae of younger ages, who started eating when transferring them to heat. Further laboratory observations of the larvae collected on boxwood plants during the winter showed that they molt another three to four times (less often five) and then pupate (Fig. 1 b, c; Fig. 2 a, b). The size of the larvae before pupation in most cases was 2.6-3.4 cm (maximally reached 4.0 cm), the size of the head capsule reached 2.5-3.0 mm. However, it was noted that the larvae before pupation could reach and only 2.2-2.4 cm in length. As a rule, the larvae of the wintering generation have the smallest sizes before pupation. Summer generations are most often significantly larger. Again, this also depends on humidity – the worse the quality of the feed, the smaller the larvae.

a b c

Figure 1. Larvae of *Cydalima perspectalis* of various instars (a – wintering larvae of the youngest instars, collected from *Buxus sempervirens* plants in January-February, b & c – larvae of the older instars), Republic of Moldova, 2020.

a b c d

Figure 2. Stage of puppies of *Cydalima perspectalis* (a – larvae in the woven cocoon, b –pupa in the first hours after molting, c & d – side and top view of pupa)

The newly formed pupa is light green, attached (not always upside down, often just lying in a woven cradle) with a cobweb to the boxwood branch (Fig. 2 b-d). Most often, before pupation, the larva weaves a dense cocoon of leaves. However, if the foliage is strongly damaged, the larva can be fixed before pupation with the help of a twisted cocoon between any branches. The length of spring pupae reached 2.2-2.3 mm.
Over time, the cocoon changes its color and, depending on the morphs of the imago, acquires its characteristic peculiar color (Fig. 4, a-e). The cocoon is attached by a web to the leaves of the plant, in the place of attachment the head capsule of the larva remaining after molting is visible. At a temperature of +22…+24 ^{o}C, pupal development continued 12±1.0 days. With a decrease in temperature to +15…+18 ^{o}C, the stage of development of the pupa increased to 17-20 days or more. Our data are similar to those obtained previously by other authors (Göttig, 2017; Nesterenkova et al., 2017). Thus, according to Göttig (2017) the pupal stage at a temperature of +25 ^{o}C lasts 9±0.5 days, at +20 ^{o}C – 17±1.0 days; according to the Nesterenkova (2017) the pupal stage at a temperature of +23 ^{o}C lasts 9 days. We assume that the development of pupae depends not only on the sum of the effective temperatures and photoperiod, but also on the quality of the feed consumed by the larvae before pupation.
It is well known that *C. perspectalis* populations have several different types of wing coloration. White wings with a dark brown margin and small characteristically crescent-shaped white marks on it characterize representatives of the typical, most common morph (Fig. 5, c-d). The body of the imago is white with a brownish abdominal segment. The melanic morph, has both a body and wings of almost the same dark brown color, with the exception of two white marks (Safian & Horvath, 2011; Szekely, 2011 & Göttig, 2017). In addition, some authors note that exists an intermediate phenotype with an extra brown margin at the forewings, which is also recognized in China (Pan et al., 2011). The moldavians' populations also have several different types of wing coloration – typical (white), intermediate and melanic morphs (Fig. 5, a-d). Our studies have confirmed the opinion of other authors (Göttig, 2017) that melanic morphs can be identified already at the pupal stage (Fig. 4, c-e).

a b c d e

Figure 3. Pupae of *Cydalima perspectalis* before the imago fly (a, b, c – top, side and bottom view of the cocoon white morphs, d – top view of the cocoon intermediate morphs, e – side view of the cocoon dark morphs

a b c d

Figure 4. Imago of *Cydalima perspectalis* top and bottom view (a, b – female intermediate morph, c, d – male typical white morph)

The moths of *C. perspectalis* are active at twilight, lead a nocturnal (and crepuscular) lifestyle. The life cycles these pest occurs completely on *Buxus* plants, were oviposition, larval development, pupation and overwintering take place. Basically, in nature, females lay eggs on overleaf. It is very difficult to find eggs on the boxwood branches, as freshly laid flat eggs are pale green, almost transparent, with a diameter of only about 1 mm. With advancing age, the eggs turn yellow, a dark head of a developing larva begins to shine through the shell. In the laboratory, egg laying can occur not only on the back side of the green leaves of the *B. sempervirens*, but also on the upper side of the leaf, as well as on other objects in the cages. The number of eggs in a clutch varies from a few to several tens, and depends on many factors – on the age of the females *C. perspectalis*, and on the conditions of rearing and feeding. In natural egg-laying, there was also a large discrepancy in the number of eggs, but in most cases it was from about 8-13 to 17-20 eggs in a clutch (Fig. 5, a). The duration of the egg stage in the laboratory was 3-5 days, in the nature its depended on the temperature conditions. Hatching larvae reach about 1.5-2 mm in length (Fig. 5, b, c). The duration of larvae development since hatching from eggs in the laboratory conditions ranged from 20 to 36 days, depending on the generation and sum of effective temperatures.

a b c

Figure 5. Eggs and new hatching larvae of *Cydalima perspectalis* in the laboratory conditions (a – ovipositions, b, c – hatching of larvae and newly hatched larvae)

Larvae of the youngest instars feed aggregated, causing damage on the exterior leaf layer. Later they spread over the plant, weave a cocoon and feed separately, typically sparing the vein and often additionally attacking the bark. Different authors point to a different number of instars for larvae *C. perspectalis* – from six to eight, in depending on the host species *Buxus* plants (Maruyama and Shinkaji, 1991; Göttig, 2017; Nesterenkova et al., 2017). We counted six and seven instars in the laboratory, when feeding the larvae with *B. sempervirens* plants. However, we believe that the number of moults may vary depending on external factors, and maybe for other reasons. Our observations have revealed that if larvae were collected from December to February in third instar, then under laboratory conditions they molted another three to four times. We also revealed a dependence of the development (however, as in many species of insects) of all stages *C. perspectalis* from egg to pupa on temperature. The lower the temperature, the longer the development time of each stage. With a decrease in temperature from 30 to 15 ^{o}C, the duration of the larval stage increases by more than 4 times, eggs and pupa stages – two to three times. Consequently, our studies of the development of *C. perspectalis* eggs, larvae and pupa showed a pronounced temperature dependence, which is confirmed by other authors (Maruyama and Shinkaji 1987; Leuthardt, 2013).

The Box tree pyralid *C. perspectalis* is an herbivorous insect that is highly monophagous and specialized on various species of plants of the genus *Buxus* – *B. microphylla*, *B. microphylla* var. *insularis*, *B. m.* var. *japonica*, *B. m.* var. *faulkner*, *B. sempervirens*, *B. sempervirens* var. *sempervirens*, *B. s.* var. *rotundifolia*, *B. s.* var. *argenteovariegata*, *B. s.* var. *aureovariegata*, *B. sinica* (Maruyama, 1992; Maruyama and Shinkaji, 1991, 1993; EPPO, 2011; Leuthardt and Baur, 2013; Leuthardt et al., 2013; Plant et al., 2019). Although some authors point to damage by larvae and some other plant species – *Euonymus alatus* (Thunb.) Siebold, *E. japonicus* Thunb. (Celastraceae), *Ilex chinensis* Sims (Aquifoliaceae) and *Murraya paniculata* (L.) Jack (Rutaceae) (Bury et al., 2017). Our observations over five years have shown that in the conditions of Republic of Moldova, the phytophagus has so far been found only on *B. sempervirens* plants. We found several foci of infection of the *B. sempervirens* plants with a larvae

C. perspectalis (Fig. 6, a-c). For five years, various degrees of infection were observed in different places, with 3 overlapping generations. As a rule, the beginning of the flight month of the first generation in the conditions of the Republic of Moldova occurs in May, the second – at the end of June-July, and adults of the third generation appear in August-September.

a b c d

Figure 6. Damage caused by larvae of *Cydalima perspectalis* to the *Buxus sempervirens* plants (a – by larvae of younger instars c – by larvae of older instars, d – irreparable damage), mun. Chisinau, Republic of Moldova, 2018-2019.

Within three years after the pest was found in the Republic of Moldova, in those foci of infection where at least several treatments were not performed during the spring-summer season, the phytophage completely destroyed boxwood plants, which subsequently dried up and almost completely died (Fig. 6 d). With timely chemical treatments, the phytophage abundance was significantly suppressed, which kept the plantations of the *B. sempervirens* plants in excellent condition. At the same time, it was established that when viral preparations were used in combination with sex pheromone traps, over time, both the number of the pest density decreased and the number of various entomophagous accumulated on the plants, and as a result, there is a death of about 10-20% of the *C. perspectalis* larvae from diseases, probably of viral origin.

Until now, it is not clear how the pest entered the territory of the Republic of Moldova. Many authors in their scientific papers put forward the hypothesis that the phytophage was introduced with plant material (Leuthardt et al., 2010; van der Straten and Muus 2010; John and Schumacher 2013; Gninenko et al., 2014). We also do not exclude this possibility. Moreover, in the Republic of Moldova there is a fairly wide range of imported plants, which are very popular in landscaping the territory of private companies, various enterprises and organizations, as well as private sites.

At the same time, a number of authors (Plant et al., 2019) indicate good flying qualities of *C. perspectalis* imagoes. And also, the species probably to be capable of bearing fairly low temperatures and spreading naturally in the continent (Feldtrauer et al., 2009; Muus et al., 2009; Bakay and Kollar, 2018). It is possible that climate change and human activities are associated with the independent expansion of many species of insects. And *C. perspectalis* is not an exception, but one of the species that have independently reached Moldova. Unfortunately, due to

its small economic importance, the species may be left without due attention from various services and institutes of plant protection. And it is not known in the future what will be the fate of boxwood, used for decorative and landscaping purposes.
Some authors (Buchsbaum and Segerer, 2013) do not consider the invasion of the *C. perspectalis* to be an event requiring urgent measures and special attention, since boxwood is not an autochthonous plant for many regions of Europe. They consider this only enrichment of biota. It is difficult to say who is right in this matter. However, we believe that monitoring populations in the conditions of the Republic of Moldova is relevant and of interest both from a scientific point of view and from a practical point of view, for managing population density and containing damage to boxwood ornamental stands.

CONCLUSIONS

In the conditions of the Republic of Moldova pest *Cydalima perspectalis* development on the *Buxus sempervirens plants* in 3 generations. Imago fly from May till September-October. Moldovan populations box tree moth, as well as all the other populations described earlier from Asia and Europe, have several different types of wing colouring – typical (white), intermediate and melanic morphs. Phytophage overwinters mainly at the stage of larvae of 3-rd instar, but it is able to hibernate in the climate of the Republic of Moldova at the second instar. The duration of the egg stage at the optimal temperature +25±2 ^{o}C consist in average 3-5 days, larval development – from 20 to 36 days, pupal stage continued 12±1.0 days. The development of all stages depends primarily on the sum of effective temperatures, as well as on other factors (host plant, etc.). With decreasing temperature, the duration of each stage increases, with increasing (up to a certain limit) it decreases. Consequently, climate change undoubtedly contributes to an increase in the number of populations of the invasive harmful species. To control the number of pests, it is necessary to carry out several treatments with systemic insecticides per season. If it is impossible to use chemical preparations, preference should be given to pheromone traps and microbiological preparations, especially of viral origin.

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YIELD OF GRAIN LEGUMES INTERCROPPING WITH CEREALS IN THE FLORINA AREA IN GREECE

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ABSTRACT

Intercropping of two or more crops in the same area at the same time is an old and commonly used practice that results in the highest overall yield on a given plot, because ofdifferent root growth, height and nutrient requirements of the crops involved. The aim of this study was to determine the possibilities of intercropping grain legumes with cereals for food production for human consumption. The completely randomized design was applied with three replications and the experiment was established in the farm of the Western Macedonia University in Florina (Greece). Particularly, lentils (two varieties) was used as grain legumes, and bread wheat (two varieties) and oat were used as cereals, which were grown individually as well as intercropped with each other in mixed rows in a sowing ratio 50:50. The plots consisted of seven rows five meters long of which the five inner were harvested. A total of 33 experimental plots was installed. The field was fertilized only with base fertilization. During the growing season, the following morphological traits were measured: height and total height, the blooming, as well as the grain yield, the length of spike (cm) and the number of fertile grain/spike. Differences were found between treatments regarding yield as well as the agronomic traits.In most cases the mixtureshad higher yield compared to their respective monocrops.

Key words: *intercropping, yield, monocropping.*

INTRODUCTION

Legumes are important crops because their seed is considered to be high-nutrient food, due to its high content of proteins (30-35%) - proteins of high biological value -, calcium and phosphorus minerals and vitamins, which are considered essential for human'snutrition. Intercropping of most annual legumes with winter cereals is a very common cultivation practice in many countries (Qamar et al. 1999; Clergue et al. 2005). Intercropping of legumes with cereals contributes to the improvement of soil properties, in weed control and in lodging resistance, important trait for some cereals. (Banik et al. 2006; Wang et al. 2012). However, it has been reported that competition of intercropped plants for moisture, light and

inorganic minerals usually results in yield reduction of the mixturescompared to monoculture (Akter et al. 2004; Dusa and Roman 2009; Lithourgidis and Dordas 2010; Menber et al. 2015).Grain legumes, especially lentil, are a food with high nutritional value, which are grown mainly for human consumption and considered an essential part or more specificallythe basis of the Mediterranean diet. Intercroppingcereals withgrain legumes could be an interesting new proposal, whichinvestigates the possibility of intercropping cereals with grain legumes for simultaneous seed harvest, in order to be applied in organic agricultural system.
The research was undertaken to study the behavior of some field crops in intercropping, and to determine the possibilities of intercropping grain legumes with cereals for food production for human consumption under the special climatic conditions of the Florina area.

MATERIALS AND METHODS

A field experiment was carried out in the farm of the University of Western Macedonia in Florina (Greece), the cultivation season 2019-2020, intercropping Greek varieties of grain legumes with cereals. Particularly, two varieties of lentils (*Lens culinaris*) was used as grain legumes, and two varieties of bread wheat (*Triticum aestivum*) and one variety of oat (*Avena sativa*) were used as cereals, which were grown individually as well as intercropped with each other in mixed rows in a sowing ratio 50:50. Thus 11 different treatments were created and a total of 33 experimental plots was installed. The examined genotypes were sown in early November 2019 in a sandy loam soil. The plots consisted of seven rows five meters long of which the five inner were harvested. The distances between rows were 0.25m. The completely randomized design with three replications was used. The field was fertilized only with base fertilization, so that 80 and 40 kg ha^{-1}, Nitrogen and P_20_5 respectively were added into the soil. The crop was kept free of weeds by hand hoeing when necessary. During the growing season, the following morphological traits were measured: height and total height, the blooming, as well as the grain yield,the length of spike or panicle (cm) and the number of fertile grain/spike.Specifically, the height of the plants was measuredin early April, and one day before the harvest.Lentils varieties used are reptiles, therefore the measure of the final height did not take place. Data were statistically analyzed and the means were compared according to LSD test at p=0.05.

RESULTS AND DISCUSSION

Intercropping had significant effects on grain yield. Specifically significant differences were recorded between the examined genotypes in yield (significant differences at p=5%, Table 1). Yield ranged from924Kg/ ha in lentil 2 (second variety) to 11963.24Kg/ha in bread wheat 2 (second variety) intercropped with lentil 2 (Table 1). Both bread wheat varieties produced more yield intercropped with both lentil varieties. The superiority of bread wheat intercropping with lentils was more obvious when the second lentil variety was involved. Intercropping increased the yield of oat as well. These results were in disagreement with those of other researchers who reported that the yield of sole crops was higher compared to their intercrops (Yagmur and Kaydan 2006, Dușa and Stan 2013). The grain yield of lentil varieties was affected less than the cereals involved in intercropping system. In intercropping with wheat, the lentil 1 yield was smallerthan in sole cropping but this difference was not statistically significant.On the contrary the lentil second variety had higher yield intercropped with cereals (bread wheat or oat). The above results suggest that there was a positive effect of intercropping to the crops involved but this effect was clearerfor the cereals rather than legumes.

Table 1. The grain yield, the height after tillering and the final height of intercropping crops and monocropping

Genotype	Grain yield Kg/ ha (for cereals)	Grain yield Kg/ha (for lentil)	Height after tillering (for cereals)	(for lentil)	Final height (for cereals)
Bread wheat 1	5111.27e		44.03		75.60cd
Oat	4028.20f		38.40		89.63b
Bread wheat 2	5293.77e		44.76		64.80e
Lentil 1		1436.53h		27.20	
Lentil 2		914.46i		27.37	
Bread wheat 1+ Lentil 1	8484.64d	1221.92h	47.03	31.57	78.23c
Bread wheat 2+ Lentil 1	11963.24b	1151.02h	48.90	31.53	63.60e
Bread wheat 2+ Lentil 2	14170.4a	2652.58g	45.53	31.73	68.23de
Bread wheat 1+ Lentil 2	10387.52c	1563.7h	45.33	31.73	82.60bc
Oat+ Lentil 1	8592.56d	2988.06fg	39.40	26.17	100.63a
Oat+ Lentil 2	5847.12e	1983gh	39.23	28.03	98.67a

Means in columns followed by different letters are significantly different at $p<0.05$ by LSD test.

The height after tillering ranged from 38.4 in sole oat to 48.9 in bread wheat 2 when intercropped with lentil 1 (Table 1). Regarding the final height the differences were significant (Table 1). The final height ranged from 63.6 in bread wheat 2 intercropping with lentil 1 to 100.63 in oat intercropped with lentil 2, followed by the oat intercropped with the other lentil' s variety. The final height of both varieties of bread wheat was not affected by the intercropping. On the contrary the final height of oat intercropping with lentil was higher compared to the sole crop.

Table 2. Blooming, length of spike, number of fertile grains/spike,fertile grains /pod of intercropping crops and monocropping

GENOTYPE	Blooming (days to heading) for cereal	Blooming (days to heading)for lentil	Length of spike, or panicle (cm)	Number of fertile grains/spike grains/panicle	Numbe r of fertile grains/ pod
Bread wheat 1	181a		7.14	22.87ab	
Oat	182a		16.36	17.5b	
Bread wheat 2	183a		8.43	22.3ab	
Lentil 1		176b			1.67ab
Lentil 2		177b			1.33ab
Bread wheat 1+ Lentil 1	181a	177b	7.92	22.57ab	1b
Bread wheat 2+ Lentil 1	180a	178b	8.37	20.77b	1.33ab
Bread wheat 2+ Lentil 2	180a	175b	8.66	21.93ab	2a
Bread wheat 1+ Lentil 2	182a	176b	8.17	27.97a	1.33ab
Oat+ Lentil 1	180a	177b	17.58	23.5ab	1b
Oat+ Lentil 2	181a	176b	17.72	22.97ab	1.33ab

Means in columns followed by different letters, are significantly different at $p<0.05$ by LSD test.

The highest number of fertile grains/spike was found in bread wheat (first variety) intercropped with lentil 2 (27.97) (Table 2). The number of fertile grains/panicle was also higher in oat intercropping with both varieties of lentil (Table 2). These results were in disagreement with the ones of Dusa and Stan (2013) who reported that the number of fertile grains/panicle was lower in intercropping, especially for the oat plants intercropped with lentil. The smallest number of fertile grains/pod was produced when lentil variety 2was intercropped with bread wheat 1, whereas the greatest number was recorded when lentil variety 2 was intercropped with bread wheat 2.The above results suggest that the behavior of intercropped species is influenced by the genotype of species involved in the intercropping system.

CONCLUSIONS

It was concluded that concerning the grain yield the mixture bread wheat 2 + lentil 2 is more productive followed by the mixture of bread wheat 2 with the other lentil variety. Concerning the mixture oat +lentil, intercropping increased the yield of oat, especially when the first variety of lentil was involved. A first estimate of these results leads to the conclusion that the genotype of species involved in the intercropping system plays an important role in the behavior of intercropped species. However further research, including different spatial arrangement of the individual crops and different cultivars, is needed to confirm the results of the present study.

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Review paper

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PASTORALISM IN THE MAGHREB: A REVIEW ON ENVIRONMENTAL, SOCIO-CULTURAL, ECONOMIC AND POLITICAL ASPECTS

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ABSTRACT

Pastoralism is a livelihood system based on extensive production of livestock (e.g. cattle, sheep, goats, camels) mainly on marginal lands. It is a traditional activity in the Mediterranean in general and the Maghreb (viz. Algeria, Morocco, Tunisia) in particular. This review casts light on research regarding pastoralism in the Maghreb. In particular, the paper analyses the benefits of pastoralism as well as the challenges faced by pastoralists in the region from the environmental, socio-cultural, economic and political points of views. A search performed in July 2020 on the Web of Science yielded 113 documents and 68 of them were included in the systematic review. The analysed literature emphasizes the negative impacts of pastoralism and the challenges faced by pastoral communities in the Maghreb. These include climate change, land degradation and desertification, poverty and livelihood vulnerability as well as the ongoing erosion of pastoral culture and traditions. Doing so, scholars question the future of pastoralism in the region and highlight the need for its adaptation and transformation through, among others, moving towards agro-pastoral systems. There is a dearth of articles that highlight the positive impacts and benefits of pastoralism in the Maghreb. However, the literature shows that pastoralism has a long tradition and is an integral part of the Maghrebi culture and history, and values the traditional knowledge of pastoralists as well as their adaptive capacity. The review shows that there is a gap in research on pastoralism in the Maghreb especially regarding economics. In this context, regional projects such as PACTORES (Pastoral ACTORs, Ecosystem services and Society as key elements of agro-pastoral systems in the Mediterranean) result crucial to bridge the current knowledge gap and foster the sustainable development of pastoralism in the Maghreb and the Mediterranean at large.

Keywords: *agro-pastoral systems, sustainability, Mediterranean, Algeria, Tunisia, Morocco.*

INTRODUCTION

Pastoralism is as old as the Mediterranean civilisations. However, the opinions about its roles in the current Mediterranean society diverge not only among scholars but also among other stakeholders. Some consider that agro-pastoral systems (APS) are marginal systems of low economic productivity, while others highlight the notorious multifunctional nature of APS and their role in the provision of multiple ecosystem services (ES). In fact, APS produce high-quality products, valorise marginal rangelands, protect biodiversity, control soil erosion and land degradation, and preserve long-standing traditions (Bewsell & Dake, 2008). Pastoralism also plays a role in climate change mitigation/adaptation and achievement of food security (Rivera-Ferre & López-i-Gelats, 2012). Furthermore, APS are central in cultural identities in the Mediterranean and create income-generating activities in marginal, remote territories. In this context, some scholars argue that pastoralism is disappearing due to different internal and external factors such as climate change, unfavourable environmental conditions, and spatial and political marginalization (Jónsson, 2010). Indeed, FAO (2020) puts that "*In spite of their social, economic, and environmental contribution to their communities, pastoralists are often misunderstood and excluded from policy processes affecting them*". Pastoralists face different emerging issues such as land grabbing, climate change and demographic changes, which affect their mobility as well as the access to and use of rangelands (Davies et al., 2014). Other scholars assert that APS, as complex adaptive systems, are suited to changing environmental and climatic conditions (Davies & Nori, 2008; Nori, 2017). Pastoralism and agro-pastoralism are traditional activities in the Maghreb (viz. Algeria, Morocco, Tunisia) (Maurer, 1992) and play an important socio-economic role (Dutilly-Diane, 2007). However, the sector faces several challenges such as rangelands degradation (Abdelguerfi & Laouar, 2000; Rocha Correa, 2013). In this respect, scientific evidence and data are crucial to highlight the multiple roles of pastoralism. For instance, FAO (2020) stresses that "*To increase pastoralism's recognition, data production is crucial for evidence-based policy-making. The collection of data is essential to understand the importance of pastoralism, its contribution to local and national societies and to better inform policies*". Therefore, this review casts light on research regarding pastoralism in the Maghreb. In particular, the paper analyses the benefits and challenges from the environmental, socio-cultural, economic and political points of views.

METHODS

The article draws upon a systematic review of all documents (journal articles, book chapters, conference articles) indexed in the Web of Science. A search was performed on 17 July 2020 using the search query: *(pastoralism or pastoralist) AND (Maghreb OR "North Africa" OR Algeria OR Morocco OR Tunisia)*. The search yielded 113 documents. Two inclusion criteria were considered: geographical coverage (viz. dealing with at least one of the three Maghreb countries) and thematic focus (viz. pastoralism). Following the scrutiny of titles, abstracts and full-texts, 45 documents were excluded. Therefore, 68 documents were included in the systematic review (Table 1).

Table 1. List of the selected documents.

Year	Number	References
2020	2	Broodbank & Lucarini (2020); Duffy (2020)
2019	4	Dressler et al. (2019); Guerin (2019); Vidal-Gonzalez & Mahdi (2019); Zerboni & Nicoll (2019)
2018	7	Boussaid et al. (2018); Brass (2018); Dunne et al. (2018); Gobindram et al. (2018); Merrills (2018); Rignall & Kusunose (2018); Vidal-Gonzalez & Nahhass (2018)
2017	10	Blanco & Michon (2017); Blanco et al. (2017); Campbell et al. (2017); Dominguez (2017); Faye et al. (2017); Gaouar et al. (2017); Giuliani et al. (2017); Mebirouk-Boudechiche et al. (2017); Volpato & Di Nardo (2017); Volpato et al. (2017)
2016	7	Ben Hounet & Guinand (2016); Ben Hounet et al. (2016); Davis (2016); Jemaa et al. (2016); Martin et al. (2016); Mebirouk-Boudechiche et al. (2016); Roubet & Amara (2016)
2015	5	Abdallah & Souilmi (2015); Blanco et al. (2015); Leder (2015); Mebirouk-Boudechiche et al. (2015); Müller et al. (2015)
2014	4	Aouadi et al. (2014); Freier et al. (2014); Ménard et al. (2014); Volpato & Howard (2014)
2013	5	Benmoussa (2013); Daoudi et al. (2013); Gamoun (2013); Linstädter et al. (2013); Muigai & Hanotte (2013)
2012	4	Akasbi et al. (2012); Chehma & Abdelhamid (2012); Dunne et al. (2012); Freier et al. (2012)
2011	1	Freier et al. (2011)
2010	3	Kuhn et al. (2010); Linstädter et al. (2010); Rössler et al. (2010)
2009	3	Le Roux & Bouazid (2009); McGregor et al. (2009); Pereira et al. (2009)
2008	1	Haddouche et al. (2008)
2007	3	Davies & Hatfield (2007); Dutilly-Diane (2007); Dutilly-Diane et al. (2007)
2006	3	Davis (2006); Roubet (2006); Smith (2006)
2005	2	Davis (2005); Le Cuziat (2005)
2004	1	Davis (2004)
2003	1	Roubet (2003)
1996	2	Benazzouz (1996); Chiche (1996)

RESULTS AND DISCUSSION

Environment

Climate change is a recurrent theme in the literature on pastoralism in the Maghreb (Blanco & Michon, 2017; Duffy, 2020; Jemaa et al., 2016; Kuhn et al., 2010). However, many pastoralists have been able to adapt to rainfall variability and droughts (Blanco et al., 2017; Daoudi et al., 2013), especially mobile pastoralists (Akasbi et al., 2012; Dressler et al., 2019; Kuhn et al., 2010). Freier et al. (2014)

indicate that the "*promotion of mobile pastoralism in semi-arid areas is a valuable option to increase resilience against climate change*" (p. 917) in southern Morocco. However, the genetic erosion of local breeds (Gaouar et al., 2017) may reduce herds' adaptability to harsh environmental and climatic conditions.

Other articles focus on the impacts of land use changes on pastoralism (Davis, 2004; Duffy, 2020; Jemaa et al., 2016; Kuhn et al., 2010; McGregor et al., 2009; Rignall & Kusunose, 2018). In particular, they highlight that commercial agriculture development decreased mobile pastoralism (cf. transhumance) (Benmoussa, 2013; Duffy, 2020; Haddouche et al., 2008; Jemaa et al., 2016). Some authors point out pastoralism as a cause of land degradation and desertification (Benazzouz, 1996; Boussaïd et al., 2018; Gamoun, 2013; Haddouche et al., 2008; McGregor et al., 2009; Zerboni & Nicoll, 2019), and deforestation (Campbell et al., 2017; Chehma & Abdelhamid, 2012; Haddouche et al., 2008; McGregor et al., 2009; Mebirouk-Boudechiche et al., 2016, 2015). However, Blanco et al. (2015) conclude that "*Saharan agro-pastoralism activities are not necessarily incompatible with acacia tree conservation, contrary to the commonly admitted postulate in Morocco*" (p. 21). Likewise, Davis (2005) highlights that "*The existing data from southern Morocco […] do not support the claims of widespread desertification due to […] pastoral activities*" (p. 509). Zerboni and Nicoll (2019) stress that "*human and herd animal activities affected geomorphic surfaces that affected slope stability, intensified erosion and dust mobilization, and enhanced dust export from the African continent offshore*" (p. 22). Many of the negative impacts on land resources are due to overgrazing (Boussaïd et al., 2018; Gamoun, 2013; Haddouche et al., 2008; Zerboni & Nicoll, 2019) that is exacerbated by pastoralism modernisation and intensification (Boussaïd et al., 2018; Vidal-González & Nahhass, 2018). However, Davis (2006) argues that "*Land degradation in the dryland agricultural areas of Morocco is commonly blamed on overgrazing by local pastoralists despite existing documentation that suggests instead that ploughing of marginal lands and over-irrigation are the primary drivers of land degradation in the region*". Linstädteret al. (2010) argue that pastoral land use management in the Moroccan High Atlas mitigates the effects of droughts, maintains the capacity of the rangeland vegetation to buffer rainfall variability, and slows down land degradation. Other authors indicate the role of pastoralists in the overexploitation of groundwater resources especially in arid Saharan areas (Boussaïd et al., 2018). The depletion of pastoral resources as a result of frequent droughts and decrease in the grazing area are among the main challenges faced by pastoralists (Gobindram et al., 2018). The degradation of pastures and rangelands increasingly lead pastoralists to provide feed supplements (Mebirouk-Boudechiche et al., 2017; Müller et al., 2015). However, Müller et al. (2015) argue that the application of supplementation is controversial, as it "*allows smallholders to avoid a breakdown in animal numbers in times of drought*" but "*keeps herd sizes high and may thus result in rangeland degradation in the long term*" (p. 153).

Society and culture

Pastoralism is an integral part of the traditions in the Maghreb. In fact, many authors analyse the history of this practice in the region (Aouadi et al., 2014; Brass, 2018; Broodbank & Lucarini, 2020; Duffy, 2020; Dunne et al., 2018; Dunne et al., 2012; Guerin, 2019; Leder, 2015; McGregor et al., 2009; Merrills, 2018; Muigai & Hanotte, 2013; Pereira et al., 2009; Roubet, 2003, 2006; Roubet & Amara, 2016; Smith, 2006; Vidal-Gonzalez & Mahdi, 2019; Zerboni & Nicoll, 2019) starting with livestock domestication (Brass, 2018; Broodbank & Lucarini, 2020; Zerboni & Nicoll, 2019). Archaeological records show different features associated with pastoralism, transhumance and herding (e.g. trails, footholds, trackways, stables, rock-shelters) (Zerboni & Nicoll, 2019), and rock engravings/painting sites (Roubet & Amara, 2016).

The literature also highlights the huge local knowledge of pastoralists (Ben Hounet et al., 2016; Davis, 2005; Gobindram et al., 2018; Linstädter et al., 2013). For instance, Gobindram et al. (2018) stress that the local ecological knowledge (LEK) of shepherds includes "*recognising and naming forage plants and rangeland types, identifying preferred or less preferred plants or plant parts, describing circumstantial palatability of plants*" (p. 207). Other authors (Davis, 2005, 2016) warn of the ongoing erosion of pastoral indigenous knowledge. In this respect, Davis (2005) argues that "*expert knowledge is based on questionable evidence and that it has been privileged over local knowledge primarily for political, economic and administrative reasons*" (p. 509). The modernisation of pastoralism also affected the culture of pastoral communities (Dominguez, 2017; Vidal-González & Nahhass, 2018) but opinions diverge on its impacts. In this sense, Vidal-Gonzalez and Nahhass (2018) argue that the use of mobile phones improved social cohesion among nomadic populations in eastern Morocco.

Some authors focus on the poverty and vulnerability of pastoral households and communities in the Maghreb (Davies & Hatfield, 2007; Freier et al., 2014; Martin et al., 2016). Indeed, the livelihood security of pastoral households is threatened by, among others, changing climate and recurrent droughts (Martin et al., 2016). Martin et al. (2016) show that drought has forced some pastoral households to abandon their transhumant lifestyle in Morocco's High Atlas. Referring to Northern Algeria, Daoudi et al. (2013) put that "*In the steppe,[…], the increase of rainfall variability augments the vulnerability of agro-pastoralists, particularly those weakly endowed with resources, and compromises reproduction of their farming systems*" (p. 303). Other scholars highlight the resilience and adaptive capacity of pastoralists (Blanco & Michon, 2017; Dressler et al., 2019; Jemaa et al., 2016; Linstädter et al., 2013). Indeed, the livelihoods of pastoral communities are undergoing different transformations (Davies & Hatfield, 2007; Faye et al., 2017; Freier et al., 2012; Gobindram et al., 2018; Haddouche et al., 2008; Le Roux & Bouazid, 2009; Rignall & Kusunose, 2018; Vidal-González & Nahhass, 2018) induced, inter alia, by climate change, land degradation and desertification as well as social changes (Vidal-González & Nahhass, 2018). Davies and Hatfield (2007) argue that "*Pastoralism is changing, adapting to market forces as well as* *demographic pressures, and influenced strongly by policies that still encourage sedenterisation*" (p. 100). The livelihood transformations include moving to agro-pastoralism (Blanco et al., 2017; Faye et al., 2017; Gobindram et al., 2018; Jemaa et al., 2016) and 'sedentarisation' (Blanco et al., 2017; Davies & Hatfield, 2007; Faye et al., 2017; Freier et al., 2012; Haddouche et al., 2008). Referring to Ait Arfa Guigou tribe in Morocco's Middle Atlas, Vidal-Gonzalez and Mahdi (2019) put that they were induced to "*shifting from nomadism to short-distance transhumance as an adaptation necessary to continue practicing sheepherding in their territory down to the present day*" (p. 129).

Pastoralists face different socio-cultural challenges such as rural exodus (especially of the young) (Blanco et al., 2017; Giuliani et al., 2017) and the lack of social recognition (Blanco et al., 2017). Dressler et al. (2019) point out to an increase in inequality in pastoral communities; they show that there is a kind of 'polarisation' between wealthy pastoralists (with large herds transported using trucks across large distances) and impoverished households (moving only by foot and experiencing decreasing herd sizes) in eastern Morocco. Also Dutilly-Diane et al. (2007) highlight two types of dominance in rangeland use in eastern Morocco "*some fractions select the best rangelands and are able to exclude other groups from it, others respond by ploughing extensive areas as a way to secure access of the surrounding pastures*" (p. 338).

Economics

There is a dearth of articles addressing pastoralism economics in the Maghreb. Davies and Hatfield (2007) underline some of the benefits of the 'sedentarisation' of mobile pastoralists and put that "*Sedentarisation, at least of the household, can bring potential benefits of access to services since few countries have adopted models of mobile or community delivered service provision. It sometimes also improves access to markets, and can reduce transaction costs through improved communication*" (p. 100). However, Gobindram et al. (2018) argue that the low profitability of pastoralism, with respect to crop production, makes its future uncertain in Morocco. The low pastoralism profitability might explain why Rössler et al. (2010) found that "*Remittances from migrants, as the most important source of income for the population in the marginalized rural regions, are partly used to subsidize farming or pastoral activities and are therefore crucial for the continuity of the agricultural system*" (p. 634) in the High Atlas region of Southern Morocco. Freier et al. (2014) analyse the vulnerability of income from sedentary pastoralism and mobile (transhumant) pastoralism to reduced precipitation and droughts in semi-arid, southern Morocco and found that mobile pastoralism is much less vulnerable to dryer climate than sedentary pastoralism. Indeed, the simulations of economic impacts of droughts on rangeland management performed by Freier et al. (2011) show that drought (2 years with 33% less precipitation) determine a decrease in profits from pastoralism by up to 57%. However, Dutilly-Diane (2007) stresses that pastoralism is the primary economic activity in the arid and semi-arid steppe areas, which occupy a large part of North Africa.

Policy and governance

Policies are addressed in two different ways; by analysing the current policies that shape pastoralism and/or by highlighting policy interventions needed to preserve and develop pastoralism in the Maghreb. Blanco et al. (2017) argue that agro-pastoralism in southern Morocco is threatened by current agricultural policies. In this context, Davis (2005) warns that "*In Morocco the crisis narrative of desertification has been invoked for decades to facilitate and justify policy and legal changes that have systematically disadvantaged pastoralists and damaged the environment*" (p. 509). However, the knowledge and traditions of pastoralists and agro-pastoralists can be safeguarded thanks to heritage policies (Ben Hounet et al., 2016; Ben Hounet & Guinand, 2016). Indeed, Ben Hounet et al. (2016) argue that a "*greater recognition of farmers' knowledge and their ability to identify hardy animals can ensure the sustainability of farms*" (p. 365). In this respect, Dutilly-Diane (2007) suggests that "*It is crucial to evaluate the contribution of the pastoral sector in national economies in order to influence government policies on livestock pastoralism and rangeland management*" (p. 69).

Different aspects relating to governance are addressed, especially the management of collective rangelands and pastures (Benmoussa, 2013; Dominguez, 2017; Dutilly-Diane et al., 2007; Rignall & Kusunose, 2018). While customary management of collectively-owned land is often blamed for chaotic or unsustainable land use, Rignall and Kusunose (2018) argue that customary land tenure systems have been effective in mediating transformations of land use and livelihoods in south-eastern Morocco. Also Blanco et al. (2017) put that "*The sustainability of natural resource use relies on flexible property rights, backed up by a social and cultural norm-based regulation system, that allow crop-livestock integration and landscape collective management*" (p. 111) in southern Morocco. Dominguez (2017) analyses a local land governance system (prohibiting access to some natural resources for a given period to assure their sustained use) in Moroccan High Atlas called 'Agdal' and points out that its decline is increasing social inequality and environmental degradation. Benmoussa (2013) warns that the "*The trend towards individual appropriation of tribal lands transgresses age-old norms and thus symbolises a new way of being within communal territories, part of an important process of social changes within a global context of liberalisation and globalisation*" (p. 668) and criticizes the state's encouragement of steppe areas use for agriculture in Algeria. Also, Akasbi et al. (2012) point out that "*both the customary rules and its flexible adaptation to physical constraints are generally beneficial in terms of conservation of the arid and semiarid rangeland resources*" (p. 307) in Moroccan Atlas Mountains. The literature also denotes pastoral communities' willingness to be engaged in developing their areas; in this regard, Le Roux and Bouazid (2009) highlight that "*the population has a strong desire to be assisted in the development of guidelines for environmental education initiatives that would enable them as a community to deal with desertification and land degradation*" (p. 59) in Sefiane (Algeria).

CONCLUSIONS

This paper provides a comprehensive review on pastoralism in the Maghreb. Despite its multifaceted and multifunctional benefits (environmental/ecological, social, cultural, economic), pastoralism is still perceived by some scholars and policy makers as ecologically damaging, economically unproductive, and culturally backward. Indeed, the analysed literature focuses on the negative impacts of pastoralism (e.g. land degradation, desertification, deforestation) and the challenges faced by pastoral communities (e.g. climate change, poverty, livelihood vulnerability, marginalization and social exclusion, pastoral culture erosion). Therefore, it is important to raise awareness about the role of pastoralism in the Maghreb to increase public investment in the sector. For that, a good starting point can be to devote more attention to pastoralism and pastoralists in the research agenda, in order to produce sound data and evidence to inform policies. This is particularly crucial since the review shows that there is a gap in research on pastoralism in the Maghreb. Future research should highlight the role of pastoralism in the provision of ES and public goods, and as an integral part of the cultural and social assets of the Maghrebi countries and communities. Furthermore, pastoralists have shown extensive capacities to adapt to variable climate and environmental conditions. Beyond being a simple production system, pastoralism is a livelihood and land-use system that is crucial for improving the living conditions of rural populations in remote, marginal areas. Beside research, there is also a need to reform the governance of the whole sector by, inter alia, improving the political representation of pastoral communities. In this respect, holistic approaches to agro-pastoral systems and communities, embedding the range of ecosystem and societal services they provide, are needed. The contribution of regional projects, such as PACTORES, can result instrumental in this respect as they allow exchanging good practices on pastoralism among academia, policy makers and pastoral communities in the Mediterranean.

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THE CHOICE OF CLIMATE CHANGE ADAPTATION STRATEGIES PRACTICED BY CASSAVA-BASED FARMERS IN SOUTHERN NIGERIA

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ABSTRACT

The study on choice of climate change adaptation strategies practiced by cassava-based farmers was conducted in Southern Nigeria. The following specific objectives were achieved: to ascertain the perceived effects of climate change in the study area and to determine factors influencing the choice of using climate change adaptation strategies by cassava-based farmers in the study area. Data were obtained through the administration of questionnaire to 300 randomly sampled cassava-based farmers in the study area. Data were analyzed using descriptive statistics such as mean, frequencies, percentages and inferential statistics such as Multinomial Logit Regression technique. The result revealed that farmers perceived increase in flood incidence (91.33%), drought (90.67%), high incidence of pests and diseases (55%) and low yield (50%) as the effects of climate change in the study area. Also, from the results, 58% of the farmers chose not to employ the use of climate change adaptation strategies while only 42% decided to choose using climate change adaptation options in the study area. The result also showed that age of the farmer, farming experience, gender, marital status, level of education, household size, access to credit, access to agricultural extension services and membership of association were the factors influencing the choice climate change adaptation strategies used by the farmers. The study concluded that socio-economic attributes of the farmers affected their choice of climate change adaptation strategies. Policy should be targeted at designing climate change adaptation technology to farmers as well as providing the enabling environment that would encourage them to employ it.

Keywords: *Choice, Climate Change, Adaptation Strategies, Cassava-based Farmers.*

INTRODUCTION

Agriculture is the springboard and engine of economic growth in developing countries which according to Ozor (2009) contributes about 40% of the gross domestic product and provides employment to 70% - 80% of the population.

Climate is crucial for agricultural production. However, climate is changing and is already affecting agricultural production, economy and livelihood of the population of developing countries (Kandy et al. 2006). Climate change is the most unprecedented threat in human history (Ozor et al. 2012). For instance, Henri-Ukoha, Ugwuja and Uhuegbulam (2017) re-iterated that climate and weather-related issues have impeded agricultural development in Nigeria. Zievogel et al. (2008) confirmed that climate change has affected agricultural productivity adversely. Sub Saharan Africa including Nigeria is highly vulnerable to climate change impact due to their overdependence on rainfed agriculture and low adaptive capacity (Bolaji et al. 2010). Climate change will continue to affect agricultural production, water scarcity (Liwenga, 2015) and food security adversely thereby increasing the risk of hunger by additional 80 million people in Africa and Asia by 2020 (Nwafor, 2007).

Climate change will frustrate farmers' effort to achieve food security unless adaptation measures are put in place (Adebayo et al. 2012). Moreso, as climate is a natural phenomenon, it is impossible for man to stop it, but measures can be used to reduce the effects (Singer and Avery, 2007). Mitigation and adaptation are two central issues to tackle climate change (Enete, 2014). Adaptation is one of the policy responses projected or actual changes in climate, with the goal of maintaining the capacity to deal with current future changes (Dixon, 2003). "Adaptation to climate change could be defined as an adjustment in human, ecological or physical system in response to actual and or would be stimuli or their effects, which moderate harm or exploits beneficial opportunities" (Shongwe, 2013). Adaptation includes anticipatory and reactive activities (Ifeanyi-Obi et al. 2012).

Therefore, with adaptation, vulnerability will be reduced (Rosenweig, Smith and Skinner, 2002). Several adaptation strategies are being practiced by farmers. These include changing crop variety(Saguye, 2016), soil and water conservation (Debela, 2017); diversification of livelihood activities, adjustments in farming operations (Intergovernmental Panel on Climate Change, IPCC, 2007); planting different crop varieties, changing planting dates (Maddison, 2007); increasing irrigation (Gbetibuo, 2008); change in crop cultivar (Akponikpe et al. 2010); drought resistant varieties (Mburu et al, 2015) and mixed cropping (Ndamani and Watanabe, 2006; Haji and Sani, 2014). Other adaptation practices adopted by farmers include: intercropping/multiple cropping, agroforestry, mulching, irrigation (Enete et al. 2011); tree planting, early planting, small scale irrigation and mulching (Adebayo et al. 2011).

However, the choice of using any of the climate change adaptation strategies is paramount. Enete (2014) conducted a study on the choice of climate change adaptation strategies among food crop farmers in South West Nigeria. Onubuogu and Esiobu (2014) also used multinomial logit to ascertain the trends, perceptions and adaptation options of arable crop farmers to climate change in Imo State, Nigeria. Deressa et al. (2008) analyzed the determinants of farmers' choice of adaptation methods and perceptions of climate change in the Nile Basin of

Ethiopia. Marie eta la (2020) studied farmers' choices and factors affecting adoption of climate change adaptation strategies: evidence from Northwestern Ethiopia. None of these looked at the drivers of the choice of a suitable and efficient adaptation options by cassava-based farmers in Southern Nigeria, leading to gap in knowledge which this study intends to fill. This study will help farmers, policy makers and other stakeholders to know the possible adaptation responses to suit the local needs of cassava farmers in the study area.

MATERIALS AND METHODS

The study was conducted in Southern Nigeria. The area is made up of South East, South West and South-South Nigeria. Multi-stage random sampling procedure was employed in sample selection. In the first stage, two regions, South East and South-South geo-political regions were selected purposively from Southern Nigeria based on areas where cassava farming is most predominant. In the second stage, one state each was purposively chosen from each of the two geo-political regions making two states. This was states that have upland (Abia) and riverine areas (Rivers). In the third stage, five Local Government Areas, (LGA) randomly selected from each state making 10 LGAs. Fourthly, five communities were selected from each LGA making 50 communities. Finally, six cassava-based farmers were selected from a list of registered cassava-based farmers in each community using simple random sampling. This gives a total of 300 cassava-based farmers in the study area. Primary data were obtained through administration of questionnaire, interview schedule and Focus Group Discussion (FGD). Validation of the survey instruments were done using a pilot survey where ten percent of the questionnaire were given to the respondents to fill with the help of trained enumerators who were employed in data collection. Data were analyzed using descriptive and inferential statistics. Descriptive statistics include percentages, mean and frequency distributions while inferential statistics involve the use of Multinomial Logit regression model (Greene, 2003). The model is expressed as;

$$Pr(Y_1 = j) = \frac{e ß_j . x_{ij}}{1 + \sum_{m=0}^{6} e^{ß_m x_{ij}}}, \quad j = 0,1,2,3,4,5,6 \quad \text{eqtn 1}$$

$eß_j$ is a vector of parameters that relate the socio-economic, farm and institutional characteristics X_i to the probability $Y = X_j$

Since the probability of the five climate change adaptation strategies will aggregate to one

Where,

Y denotes the random variable taking on the values of (0,1,2,3,4,5) for a non-negative integer j, while “x” denotes a set of conditioning variables. In this study, Y represents the climate change adaptation strategies while x represents the cassava-based farmers’ socio-economic characteristics. The study assumes that

probability of choosing a single climate change adaptation strategy by any cassava farmer is independent of the probability of choosing another type of climate change adaptation strategy.
The parameter estimates of the MNL model provides direction of the effect of the independent variables on the dependent (choice) variable; hence the estimates represent neither the actual magnitude of change nor the probabilities (Greene, 2000).
Where,
$Y_{i=0,1..,n} = \delta_0 + \delta_1X_1 + \delta_2X_2 + \delta_3X_3 + \delta_4X_4 + \delta_5X_5 + \delta_6X_6 + \delta_7X_7 + \delta_8X_8 + \delta_9X_9 + \delta_9X_9 + \delta_{10}X_{10} + \delta_{11}X_{11}$
Where,
Y_0 = Choice of using no climate change adaptation practice (Y = 0)
Y_1 = Choice of using improved cassava variety (Y = 1)
Y_2 = Use of minimum tillage (Y = 2)
Y_3 = Use of change in planting dates (Y = 3)
Y_4 = Mixed cropping (Y = 4)
Y_5 = Use of conservation technique (Y = 5)
The explanatory variables are:
X_1 = Age of the farmer (Years)
X_2 = Farm Experience (Years)
X_3 = Gender (Dummy: Male=1; Female =0)
X_4 = Marital Status (Dummy: Married = 1; Single = 0)
X_5 = Level of Education (Years spent in school)
X_6 = Household Size (Number)
X_7 = Access to Credit (D: Access = 1; No access = 0)
X_8 = Access to Extension (D: Access = 1: No access = 0)
X_9 = Membership of Association (D: Member = 1; Non-member = 0)
X_{10} = Farm Income (Naira)
X_{11} = Non-farm Income (Naira)

RESULTS AND DISCUSSION

Perceived Effects of climate change in the study area

Fig 1 below shows the perceived effects of climate change in the area. Majority of the cassava farmers 91.3% indicated high incidence of flood as the most perceived effects of climate change in the area. In recent times, there has been high incidence of flood recorded in Southern agricultural zones which has left millions of farm land impoverish and un-sustainable leading to poor yields in outputs and income of the crop farmers in general. Again, high incidences of pests and diseases have ravaged most of the agricultural crops including cassava plants leading to low yields and income as perceived by 54% of the cassava farmers (Osuji et al. 2017). About 43% of the cassava farmers pointed crop failure as an effect of climate change noting that a lot of farmers have experienced adverse crop failure since the emergence of climate change. More than 37% of cassava farmers perceived irregular temperature and unpredicted rainfall as vital impacts of climate change

(Braimoh et al. 2016). Temperature and rainfall have been unstable and ever fluctuating indicating a negative trend in relation to crop production. Low yields, low rain and seasonal changes accounted for 55%, 7% and 8% of the cassava farmers and were seriously noted as consequences of climate change. These variables as occasioned by climate change influences agricultural productions to a large extent deprive farmers their only source of livelihood (Shongwe, 2013).

However, less than 15% of cassava farmers experienced hunger/food security as well as high incidence of rainfall. Again, these factors have been a source of concern to majority of the farmers implying that most farm households cannot afford a three consecutive square meal per day not to talk of providing a balanced diet for their dependents. In addition, these farm households also suffered financial lack due to climatic changes and this further result to their impoverishment. Furthermore, destruction of nature 16% and poor stem survival 14% were also perceived as effects of climate change in the area. Due to the nature of most farm lands triggered by climate change, hardly viable stems survive in the soil leading to low harvest and food shortage (Buchner et al. 2017). Conclusively, 90.67% and less than 24% of the cassava farmers opted for drought and poor plant performance as negative consequences of climate change in the area.

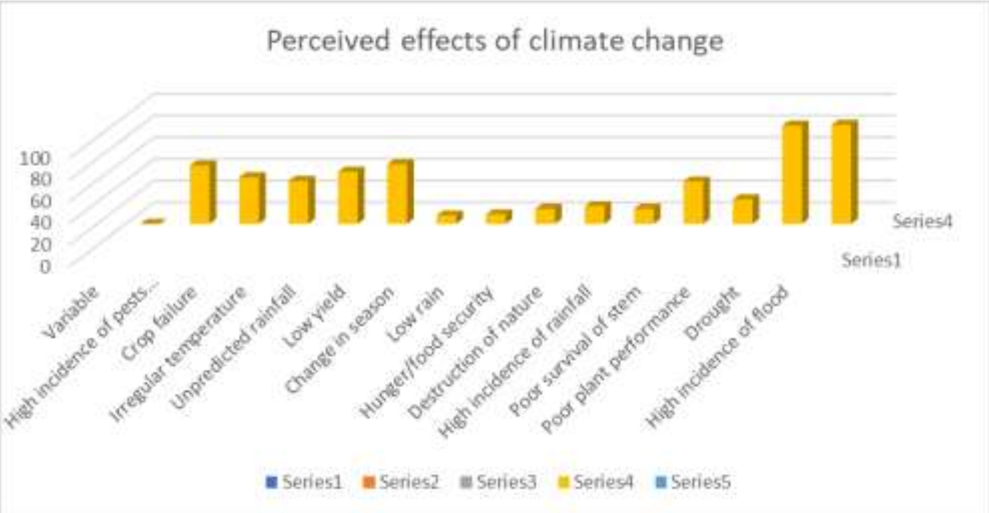

Figure 1. Perceived effects of climate change in Southern Nigeria

Choice of using Climate Change Adaptation Strategies

The cassava-based farmers' choice of using climate change adaptation strategies is shown in fig 2. From the results, majority (58%) of the farmers did not choose the use of climate change adaptation strategies while 42% of the farmers chose using climate change adaptation strategies. This indicates that a good proportion of the farmers in Southern Nigeria chose not to practice effective climate change adaptation strategies in their farms. The choice of employing climate change adaptation strategies will help the farmer to cope with the devastating effects of the changing climate, which has implications for food security in the study area. However, failure to choose the use of effective climate change adaptation strategies could be attributed to inadequate resources required to practice the adaptation technologies.

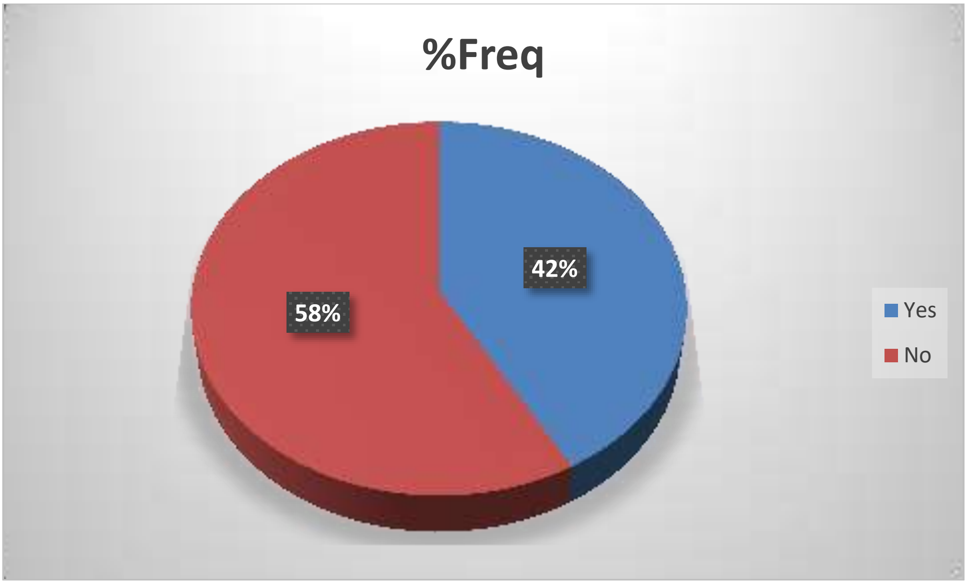

Figure 2. Farmers' choice of using climate change adaptation strategies

Result of the Multinomial Logit of factors influencing the choice adaptation strategy

Table 1. Result of the Multinomial Logit of factors influencing the choice adaptation strategy in the study area.

Variables	Use of Improved Varieties	Zero Tillage	Change in Planting dates	Use of Conservatio n Technique	Construction of drainage channels
Age	-0.2133	-0.0093	-0.3292	-0.0118	-0.0271
	(-3.3750)**	(-0.0145)	(-5.0336)**	(-0.1764)	(0.3875)
Farm	-0.0053	0.0639	0.0654	0.0671	0.0700
Experience	(-0.0501)	(2.8069)**	(2.7442)**	(2.7243)**	(0.3875)
Gender	0.9194	0.8490	0.6230	0.8224	0.5242
	(1.0934)	(3.3871)**	(0.7136)	(0.9085)	(0.5392)
Marital	0.9696	0.5035	0.2258	0.0572	0.4883
Status	(1.0229)	(3.0430)**	(1.3443)	(2.5854)*	(1.4295)
Education	0.3548	0.5307	-0.0695	-0.1051	-0.0584
Level	(2.7355)**	(4.0419)**	(-0.5223)	(-0.7745)	(4.0775)**
Household	0.5578	0.2980	0.8266	0.9716	-0.2150
Size	(2.0181)*	(0.3377)	(0.9299)	(3.1783)**	(4.5648)**
Farm Size	-3.2836	-3.5369	-1.5681	3 .0409	3 .1592

	(1.0229)	(3.0430)**	(1.3443)	(2.5854)*	(2.6202)**
Access to	1.7738	1.9292	3.0372	3.1020	1.7645
Extension	(1.2040)	(1.5968)	(2.4938)*	(2.5242)*	(1.4046)
Access to	5.1299	11.0502	11.1022	11.0961	11.4716
Credit	(2.1670)*	(0.0162)	(0.0163)	(0.0163)	(0.0169)
Membership of Association	1 .1117	-.0230	-.3962	-.2992	4.2903
	(4.6379)**	(0.0185)	(-0.3150)	(0.2650)	(3.1827)**
Farm income	11.89e- 06	7.53e-06	8.76e-06	7.11e-06	4.51e-06
	(0.0189)	(1.2264)	(1.4151)	(1.1304)	(0.6854)
Non-farm	-2.71e-06	-2.11e-06	-1.93e-06	-9.27e-07	-1.98e-06
Income	(1.2604)	(0.9679)	(0.8694)	(-4.0650)**	(-0.7952)
Constant	-4.372	-.5335	9 .6708	-6.2792	-4.4756
	(-3.2967)**	(0.1225)	(2.8374)**	(-2.5018)*	(1.6469)

Number of observations	300
LR Chi Square	37.21
Probability	0.7889
Pseudo R^2	0.0376
Log-likelihood R^2	-476.41

*Source: Field Survey, 2020

From the result in Table 2, using no adaptation as the base category, the result of the Multinomial Logit show that different farm household characteristics (age of farmer, farming experience, gender, marital status, level of education, household size; farm specific variables (farm size) and institutional variables (access to extension, access to credit, membership of association) affected farmers' choice of using climate change adaptation strategies. This is agreement with the findings of Onubuogu and Esiobi (2014) who reported that socioeconomic characteristics of farmers affected their choice of climate change adaptation strategies in Imo Sate, Nigeria. The log-likelihood chi square (X^2) is significant at ($P<0.01$) indicating that the model has a good fit.

Age of the farmer

The result shows that there is a significant negative relationship between age of the farmer and the probability of choosing the use of improved cassava varieties($P<0.01$) and change in planting dates($p<0.01$) as climate change adaptation strategies. This suggests that older farmers choose to use these adaptation strategies while younger farmers do not choose to use these climate change adaptation strategies in the study area. This implies that a unit increase in the age of cassava farmers will decrease the probability of choosing the use improved cassava varieties and change in planting dates by (21.33%) and (32.92%) respectively in the study area.

Farming Experience of the farmer

The result in Table 2 shows that there is a significant positive relationship between farming experience of the farmer and the probability of choosing the use of minimum tillage (P<0.01), change in planting date (P<0.01) and use of mixed cropping (P<0.01). This indicates that experienced farmers choose minimum tillage, change in planting dates as well as mixed cropping as climate change adaptation strategies in the study area. This implies that a unit increase in farm experience of the farmer will increase the choice of using the use of minimum tillage, change in planting date and use of mixed cropping by (63.90%), (65.40%) and (67.10%) respectively as climate change adaptation strategies.

Gender of the farmer

The result shows that there is a significant positive relationship between gender of the farmer and the probability of choosing minimum tillage (P<0.01) as climate change adaptation strategy, indicating that male farmers choose to use minimum tillage while female farmers choose not to use climate change adaptation strategies. This implies that a unit increase in the gender of cassava farmers will increase the probability of choosing use of minimum tillage by (84.90%) as climate change adaptation strategies. This suggest that gender of the households had a positive impact on farmer's decision to choose adaptation options. This implies that male-headed households had better opportunities to practice adaptation measures and access to technologies and climate change information than female-headed households, hence place them in a better position to practice diverse adaptation strategies. This result was in agreement with (Belay et al. 2017).

Marital Status

Table 2 shows a positive relationship between the marital status of farmers and the probability of choosing the use of minimum tillage (P<0.01) and mixed cropping (P<0.05) in adapting to climate change in the study area. This shows that married farmers chose to use zero tillage and mixed cropping as climate change adaptation practices in the study area. Hence one unit increment in being a married will increase the probability of choosing to use minimum tillage and use of mixed cropping by (50.35%) and (57.20%) as climate change adaptation strategies in the study area.

Level of Education

The result shows that level of education has a positive effect on the probability of choosing the use of improved cassava varieties (P<0.01), use of minimum tillage (P<0.01) and conservation techniques (P<0.01). This suggests that the number of years spent in school by the farmer influences the choice of using improved cassava varieties, use of minimum tillage and conservation techniques as climate change adaptation strategies by the cassava-based farmers in the study area. This implies that a unit increase in the number of years spent in school increase the probability of choosing and using improved cassava varieties, use of minimum tillage and conservation techniques by (35.48%), (53.07%) and (58.40%) as climate change adaptation strategies.

Household Size

From the result, household size influences the probability of choosing the option of using improved cassava varieties (P<0.05), mixed cropping (P<0.01) and conservation practices (P<0.01), suggesting that the larger the household size, the higher the chances of choosing improved cassava varieties, conservation practices and conservation practices as climate change adaptation strategies in the study area. This suggests that a unit increase in the size of the household increases the probability of choosing the option of using improved cassava varieties, mixed cropping and conservation practices by (55.78%), (97.16%) and (21.50%) respectively as climate change adaptation strategies. Household size had a positive impact on farmer's decision to choose adaptation options (Marie et al. 2020).

Farm Size

Also, from Table 2, the size of farm correlates with the probability of choosing the minimum tillage ($P<0.01$), mixed cropping ($P<0.01$) and use of conservation measures ($P<0.01$). Hence, the larger the farm, the higher the probability of choosing minimum tillage, mixed cropping and the use of conservation techniques as climate change adaptation strategies in the study area. A unit increase in farm size will increase the probability of choosing to use minimum tillage, mixed cropping and use of conservation measures (35.37%), (30.41%) and (31.59%) respectively as climate change adaptation strategies by cassava-based farmers in the study area. This implies that farmers who have large farms are more likely to take these adaptation decisions as they have enough resources to implement the effective adaptation options. This result is in agreement with the study by Kide (2014) pointed out households with relatively large farm sizes were more likely to take up new adaptation strategies when compared to farmers with small farm sizes.

Access to Extension

Table 2 shows that access to agricultural extension services increase the probability of choosing change in planting dates ($P<0.05$) and mixed cropping ($P<0.01$) as climate change adaptation strategies. This shows that the more contact cassava farmers have with agricultural extension services, the higher the probability of choosing the use of change in planting dates and mixed cropping as climate change adaptation strategies in the study area. This implies that a unit increase in access to extension will increase the probability of choosing and using change in planting dates and mixed cropping by (30.37%) and (31.03%) respectively as a climate change adaptation strategy.

Access to Credit

Table 2 also shows that access to agricultural credit increases the probability of choosing the use of improved cassava variety ($P<0.05$) as a climate change adaptation strategy. This shows that the more cassava farmers have access to credit facilities, the higher the probability of using improved cassava varieties as a climate change adaptation strategy and that farmers who do not have access to credit may not use improved variety as an adaptation strategy in the study area. This implies that a unit increase in access to credit will increase the probability of choosing and using improved cassava variety by (51.29%) as a climate change adaptation strategy.

Membership of Association

The result in Table 2 shows that membership of association influences the choice of using improved cassava varieties ($P<0.01$) and conservation techniques ($P<0.01$) as climate change adaptation strategies. This indicates the more the number of associations a farmer belongs to, the higher the probability improved cassava varieties and conservation techniques. This implies that a unit increase in membership of association will increase the probability of choosing and using improved cassava varieties and construction of drainage channels by (11.12%) and (42.90%) respectively as climate change adaptation strategies in the study area.

Non-Farm Income

Table 2 also shows that the ability to get income from non-farm sources increases the probability of choosing the use of mixed cropping ($P<0.01$) as a climate change adaptation strategy. This shows that the more cassava farmers acquire money from non-farm sources, the higher the probability of using mixed cropping as climate change adaptation strategy and that farmers who do not get funds from non-farm income sources may not use conservation technique as a adaptation strategy in the study area. This implies that a unit increase in the ability to get non-farm income will increase the probability of choosing and conservation technique by (51.29%) as a climate change adaptation strategy.

CONCLUSION

The study revealed that the cassava-based farmers perceived high incidence of flood, drought, high incidence of pests and diseases and low yield as the effects of climate change in the study area. Most of the farmers chose not use practice adaptation technologies. Also from the study, such socio-economic attributes of the farmers such as age, farming experience, gender, marital status, household size, farm size, access to credit, access to extension, membership of association and non-farm income affect the choice of climate change adaptation strategies used by the cassava-based farmers in the study area.

POLICY IMPLICATIONS

Farmers ability to choose effective climate change adaptation strategies are dependent on the such household socio-economic characteristics as age of the farmer, farming experience, gender, marital status, level of education, household size, access to credit, access to agricultural extension services and membership of association. These should therefore be taken into consideration when formulating climate change policies. Climate policy should focus on climate adaptation across gender lines, promoting awareness creation and increasing capacity building on climate change through knowledge and skill sharing platforms such as training, conferences, and seminars. Again, facilitating the availability of credit; institutional, policy, and technology support for agricultural extension services for adaptive technologies and membership of co-operatives could improve smallholder farmers' ability to spread their adaptation strategies across a range of adaptation portfolios and the level of adaptation measures.

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CHIPILÍN BIOMASS PRODUCTION OF *Crotalaria longirostrata* Hook & Arn UNDER DIFFERENT FORMULATIONS OF FERTILIZATION AND SOLAR RADIATION LEVELS

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ABSTRACT

Chipilín is a shrub species that grows wild in the tropical areas of Mexico and whose crude protein content is 31%. It is linked to food culture, mainly of the rural population. It is frequently found associated among tropical crops. Despite the fact that it is a plant genetic resource appreciated by rural families, its use continues to depend essentially on its natural reproduction and collection, perhaps because almost everything about the agronomic management of the plant is unknown. The objective of this work was to study the growth response and production of foliar biomass of *Crotalaria longirostrata* supplying different fertilization formulations (N, P and K) and different levels of solar radiation. A 3 x 3 factorial experiment was carried out, in a completely randomized design. The formulations were 45-30-26, 17-00-00 and 00-00-00, and the radiation levels were 50%, 65% and 100%, resulting in 9 treatments with 8 repetitions; and as an experimental unit a pot with a plant was considered. The results indicate significant differences ($p < 0.05$) between formulations, and solar radiation levels for all the variables considered, such as basal stem diameter, number of lateral branches, dry biomass of stem, leaf and root. In all cases the formulation 45-30-26 turned out to be better, for example at 100 days the amount of dry leaf biomass was 17.5 grams per plant, while with the formulations of 17-00-00 and 00-00 -00 was 6.0 and 3.5 grams per plant respectively. In the case of solar radiation, 15.0, 8.0 and 3.0 grams were obtained for 100%, 65% and 50% respectively. Therefore it is concluded that the best formulation and percentage of solar radiation for the production of Crotalaria longirostrata is 45-30-26 with 100% solar radiation, and that the amount of total fresh biomass that a plant can produce depends on the basal diameter of its stem.

Key words: *Phytogenetic resource, formulation, solar radiation, dry biomass*.

INTRODUCTION

Globally, plant genetic resources for food and agriculture are being lost at an unprecedented rate. Since the beginning of this century, around 75% of the genetic diversity of agricultural crops has been lost (FAO, 2016). Tropical forests are falling at a rate of just under 1% per year (Shelef *et al*, 2017) and only about 150 of an estimated 30,000 edible plant species are cultivated, and of these few species, genetic diversity has decreased even in the number of varieties marketed (Shelef *et al*, 2018; Sethi, 2015). At the same time, research is primarily focused on improving the productivity of some species of cash crops, rather than increasing crop diversity (Shand, 2000). This represents a serious loss of agrobiodiversity and the erosion of genetic diversity, leading to a food industry more susceptible to factors associated with global climate change (Sethi, 2015). Therefore, incorporating new local foods and species is a way to diversify food and income for local communities that depend on agriculture. In particular, adapting local communities to climate change will be essential for food security and poverty reduction (FAO, 2016).

Mexico is within the Mesoamerican region, considered one of the most important centers of origin and genetic diversity in the world (CONABIO, 2020; Jiménez *et al*, 2014), for which it has plant genetic resources with high nutritional and nutritional value, aspect that has been addressed by different investigations where it is shown that wild plants have a high content of nutritive elements compared to various commercial crops (Solís 2014). One of these species is the chipilín (*Crotalaria longirostrata*), which is a shrub plant that grows wild in the tropical areas of our country and whose crude protein content is 31%, high content of fiber, minerals and vitamins. (Laguna, 2016). Furthermore, it is an important food in the diet of the rural population that lives in the Mexican, Honduran and Salvadoran tropics. In the state of Tabasco and Chiapas, it is common to find the chipilín associated between tropical crops and the milpa because it is strongly linked to the culinary and food culture of the population (Maldonado, 2016; Mascorro, 2017) for being an accompaniment to food or dishes regional, such as: chipilín tamales, chipilín soup, stew with chipilín, “mondongo” and green pochitoque, to name a few. Currently, despite the fact that it is a plant genetic resource widely appreciated by rural families, its use continues to depend essentially on its natural reproduction and collection (Greenberg, 2015), the above, perhaps because almost everything is unknown in relation to management. agronomic of the plant. On the other hand, the productivity of the plants depends to a great extent on an adequate nutrition. When plant roots find nutrient availability and absorb in sufficient quantities the elements they need to perform their physiological functions, they can express their maximum potential for growth and production (Toledo, 2016). Soil is the main container of nutrients for plants; however, in most of the times, the nutrient contents are not sufficient, so it is necessary to apply fertilizers and other remedies that allow increasing soil fertility. It was considered to evaluate the effect of N, as a fundamental part of the formulations, because the high temperatures that occur in the area decrease the nitrogen content, due to the increase in the mineralization speed of organic matter, appearing simple compounds that are easily leached (Paredes, 2013). Also because the main purpose is to produce the largest amount of chipilin foliar biomass, since it is the usable part for feeding. Although legumes live in symbiosis with atmospheric nitrogen fixing bacteria, they are not enough in the case of Crotalaria longirostrata, because they only provide less than 50% of the nitrogen required by the plant, even when inoculation is carried out directly (Camarillo and Mangan 2020). Another essential element for plants to adequately carry out their physiological functions is solar radiation, since the process of photosynthesis through which it produces carbohydrates, a fundamental product for their growth and other functions, depends primarily on it. physiological. Possibly, by supplying the primary nutrients, it is possible to increase the biomass production of the chipilín, because its demand cannot be supplied by natural fertility, since tropical soils in general have low to low fertility (Molina and Meléndez, 2001). And due to its growth habit and since its requirement for solar radiation is unknown, it allows us to suppose that the management of radiation is a fundamental factor for the chipilín to have a good growth and therefore, a greater accumulation of foliar biomass. Therefore, the present study consisted of evaluating different fertilization doses and levels of solar radiation, in order to find the condition that allows obtaining good growth and the greatest accumulation of leaf biomass of the chipilin, thus contributing to the generation process of knowledge on the agronomic management of the plant, whose information will be useful for small producers who wish to adopt the technology and for its future use as a commercial crop.

MATERIAL AND METHODS

The research was carried out during the period from January to May 2015, in the Experimental Field of the UACh Southwest Regional University Unit, in Teapa, Tabasco Mexico. It is located between the coordinates 17 ° 15 'and 17 ° 45' north latitude, and 90 ° 38 'and 93 ° 46' west longitude. The predominant climate according to the Köppen classification modified by García (1988) is the Af (i ') g; warm humid with rain all year. The total annual precipitation is 3,945 mm with an annual average temperature of 26°C.

Chipilín seeds were collected from the plots of small producers in the region. These were previously treated with hot water (98^{o}C) to promote germination and were then placed in Petri dishes with wet towels. Once germinated and with 1.0 cm of radicle, two seedlings per black nursery pot were deposited with a capacity of 10 liters of soil. According to the chemical analysis of the soil, this has a pH of 5.0, 7% of organic matter, high content of nitrogen (0.32%), low content of phosphorus and potassium with 5.34 mg kg-1 and 0.06 µmol kg-1 respectively.

A full 3 x 3 factorial experiment was established in a completely randomized design. The levels of solar radiation were 100, 65 and 50% and the fertilization formulations were 45-30-26, 17-00-00 and 00-00-00, resulting in 9 treatments with 8 repetitions; and as an experimental unit a pot with a plant was considered.

The maximum and minimum photosynthetically active radiation on a sunny day in March for the different percentages of solar radiation were: 100% =2145 and 494 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 65%= 1245 and 281 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 50%= 825 and 192 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

To establish radiation levels, shade mesh covers with different shading percentages were constructed. Fertilization formulations were determined based on the rational methodology proposed by Rodríguez (1993), and using alfalfa (*Medicago sativa*) as a reference crop because it is a legume species with characteristics similar to *Crotalaria longirostrata*.

Statistical analysis was carried out with analysis of variance, Tukey's multiple comparisons test and simple linear regression.

RESULTS AND DISCUSSION

Table 1 shows the behavior over time of the production of dry and fresh leaf, stem and root biomass, as well as the basal diameter of the stem and the number of lateral branches of *Crotalaria longirostrata* subjected to different treatments. The results indicate significant differences ($p < 0.05$) between treatments for all the variables considered. In general, the best results were obtained with treatment 1 (100% solar radiation and fertilization formula 45-30-26) where the values of dry biomass at 110 days after sowing were 13.5, 26.4 and 15.3 g per plant for leaf, stem and root respectively, followed by treatment 4, with 9.9, 23.9 and 12.1 g per plant, while the worst treatments were 8 and 9, having a dry matter of 2.0, 2.6 and 1.0 g per plant for leaf, stem and root respectively. This is because, on the one hand, N is an essential element for the growth and development of plants, as it plays a fundamental role in the biochemical and physiological functions of the plant, participating in the production of chlorophyll, for the photosynthesis process, in addition to being part of several proteins that catalyze and regulate plant growth processes (Leghari et al 2016; Muñoz et al, 2013). For its part, P, helps cell division, as well as in the formation and development of roots and stems (Ocampo et al, 2012) and K, plays a role in load balance, osmotic adjustments and activation enzyme in plant cells (Wakeel et al, 2016).

In Figures 1 and 2, the effect of different levels of solar radiation and different fertilization formulations can be observed with greater precision, where the 45-30-26 formulation turned out to be better, for example at 100 days the amount of biomass Leaf dry was 17.5 grams per plant, while with the formulations of 17-00-00 and 00-00-00 it was 6.0 and 3.5 grams per plant respectively. For the percentages of solar radiation, 15.0, 8.0 and 3.0 grams were obtained for 100%, 65% and 50% respectively.

Table 1. Biomass production, basal stem diameter and number of lateral branches of *Crotalaria longirostrata*, at three levels of solar radiation and three fertilization formulations in the humid tropics of Mexico.

Factor A	Factor B	Dry leaf biomass (g)			Fresh leaf biomass (g)			Dry stem biomass (g)			Fresh stem biomass (g)		
		50	80	110	50	80	110	50	80	110	50	80	110
a1	b1	1.9 ***a***	12.2 ***a***	13.5 ***a***	8. 0 ***a***	56.8 ***a***	76.8 ***a***	1.0 ***a***	11.2 ***a***	26.4 ***a***	5.6 ***a***	68.0 ***a***	87.1 ***a***
	Sx̄	± 0.03	± 1.0	± 0.9	± 0.59	± 1.58	± 2.55	± 0.13	± 0.7	± 1.33	± 0.1	± 1.62	± 2.25
	b2	0.3 ***c***	2.6 ***c***	9.6 ***b***	1.1 ***b***	15.9 ***d***	57.3 ***b***	*0.2 c*	1.3 ***b***	22.6 ***b***	0.5 ***c***	11.8 ***c***	65.0 ***b***
	Sx̄	± 0.02	± 0.3	± 1.0	± 0.05	± 1.16	± 4.11	± *0.01*	± 0.1	± 0.99	± 0.04	± 0.67	± 2.23
	b3	0.1 ***d***	0.4 ***d***	4.6 ***c***	0.6 ***b***	24.7 ***c***	29.7 ***c***	0.1 ***c***	0.4 ***b***	8.1 ***c***	0.2 ***cd***	3.1 ***d***	19.0 ***c***
	Sx̄	± 0.03	± 0.02	± 0.25	± 0.12	± 0.55	± 1.325	± 0.01	± 0.04	± 0.7	± 0.03	± 0.5	± 1.97
a2	b1	1.4 ***b***	9.1 ***b***	9.9 ***b***	6.6 ***a***	46.3 ***b***	56.2 ***b***	0.6 ***b***	10.0 ***a***	23.9 ***ab***	4.6 ***b***	44.7 ***b***	85.5 ***a***
	Sx̄	± 0.05	± 0.55	± 1.03	± 1.07	± 1.93	± 1.86	± 0.11	± 0.72	± 1.11	± 0.1	± 4.38	± 4.11
	b2	0.1 ***d***	0.4 ***d***	1.8 ***d***	0.6 ***b***	7.8 ***e***	10.6 ***de***	0.1 ***c***	0.5 ***b***	3.8 ***d***	0.2 ***cd***	1.4 ***d***	12.9 ***cd***
	Sx̄	± 0.02	± 0.02	± 0.13	± 0.07	± 0.27	± 0.8	± 0.01	± 0.05	± 0.18	± 0.3	± 0.1	± 0.22
	b3	0.1 ***d***	0.5 ***d***	2.5 ***cd***	0.4 ***b***	3.5 ***ef***	12.3 ***de***	0.1 ***c***	0.5 ***b***	3.3 ***d***	0.1 ***d***	1.7 ***d***	11.6 ***cd***
	Sx̄	± 0.03	± 0.08	± 0.28	± 0.09	± 0.33	± 0.59	± 0.02	± 0.02	± 0.18	± 0.04	± 0.21	± 0.84
a3	b1	0.1 ***d***	0.6 ***d***	1.8 ***d***	0.5 ***b***	4.0 ***ef***	14.6 ***d***	0.1 ***c***	0.5 ***b***	3.3 ***d***	0.2 ***cd***	2.8 ***d***	12.5 ***cd***
	Sx̄	± 0.02	± 0.05	± 0.18	± 0.08	± 0.28	± 0.56	± 0.01	± 0.05	± 0.18	± 0.04	± 0.17	± 0.68
	b2	0.1 ***d***	0.3 ***d***	2.0 ***cd***	0.3 ***b***	1.8 ***f***	10.6 ***de***	0.1 ***c***	0.4 ***b***	2.6 ***d***	0.1 ***d***	0.9 ***d***	10.0 ***cd***
	Sx̄	± 0.02	± 0.05	± 0.15	± 0.13	± 0.05	± 0.65	± 0.01	± 0.04	± 0.23	± 0.15	± 0.12	± 1.17
	b3	0.1 ***d***	0.4 ***d***	1.2***d***	0.3 ***b***	2.8 ***f***	5.3 ***e***	0.1 ***c***	0.3 ***b***	1.5 ***d***	0.11 ***d***	1.1 ***d***	6.6 ***d***
	Sx̄	± 0.03	± 0.02	± 0.3	± 0.11	± 0.43	± 0.5	± 0.01	± 0.06	± 0.22	± 0.05	± 0.15	± 0.76

Continuation……

Factor A	Factor B	Dry root biomass (g)	Fresh root biomass (g)	Basal diameter (mm)			Number of lateral branches		
		110	110	50	80	110	50	80	110
a1	b1	15.3 ***a***	82.3 ***a***	6.0 ***a***	12.9 ***a***	19.8 a	15.5 ***a***	5.5 ***abc***	6.0 ***b***
	sx̄	± 0.91	± 3.02	± 0.15	± 0.98	± 1.18	± 1.9	± 0.3	± 0
	b2	5.2 ***c***	30.0 ***c***	2.3 ***c***	5.8 ***b***	11.2 b	0 ***b***	7.8 ***a***	8.0 ***a***
	Sx̄	± 0.36	± 0.5	± 0.15	± 0.3	± 0.6	± 0	± 0.3	± 0.4
	b3	2.4 ***d***	15.8 ***d***	2.1 ***c***	3.6 ***bc***	8.1 c	0 ***b***	6.3 ***ab***	7.3 ***ab***
	Sx̄	± 0.21	± 1.58	± 0.12	± 0.32	± 0.65	± 0	± 1.0	± 0.6
a2	b1	12.1 ***b***	50.0 ***b***	5.2 ***b***	11.4 ***a***	13.6 b	12.3 ***a***	6.0 ***ab***	5.8 ***b***
	Sx̄	± 0.86	± 1.9	± 0.16	± 0.91	± 0.88	± 1.9	± 0.4	± 0.5
	b2	0.8 ***d***	4.7 ***ef***	1.8 ***c***	3.7 ***bc***	7.3 c	0 ***b***	5.3 ***bcd***	6.5 ***ab***
	Sx̄	± 0.06	± 0.36	± 0.11	± 0.08	± 0.42	± 0	± 0.3	± 0.3
	b3	0.6 ***d***	2.7 ***f***	1.9 ***c***	3.6 ***bc***	5.7 cd	0 ***b***	5.8 ***ab***	7.3 ***ab***
	Sx̄	**± 0.1**	± 0.3	± 0.1	± 0.12	± 0.28	± 0	± 0.3	± 0.3
a3	b1	1.5 ***d***	9.9 ***de***	2.1 ***c***	4.0 ***bc***	5.7 cd	0 ***b***	4.0 ***bcd***	5.5 ***bc***
	Sx̄	± 0.2	± 0.65	± 0.13	± 0.26	± 0.38	± 0	± 0.7	± 0.3
	b2	1.0 ***d***	6.6 ***ef***	1.8 ***c***	3.2 ***c***	5.4 cd	0 ***b***	3.3 ***cd***	3.8 ***cd***
	Sx̄	± 0.18	± 1.05	± 0.14	± 0.32	± 0.35	± 0	± 0.3	± 0.5
	b3	0.8 ***d***	5.9 ***ef***	1.9 ***c***	2.7 ***c***	4.2 d	0 ***b***	3.0 ***d***	3.5 ***d***
	Sx̄	± 0.14	± 0.63	± 0.19	±0.21	± 0.2	± 0	± 0.4	± 0.3

Mean values with different letter indicate statistical difference ($P < 0.05$) according to Tukey's multiple range test.

Factor A: Levels solar radiation (a1=100%, a2=65% and a3=50%)

Factor B: Fertilization formulaciones NPK (b1=45-30-26, b2=17-00-00 and b3=00-00-00)

Days after planting: 50, 80 and 100

Treatments: T1=a1b1, T2=a1b2, T3=a1b3, T4=a2b1, T5=a2b2, T6=a2b3, T7=aa3b1, T8=a3b2, T9=a3b3

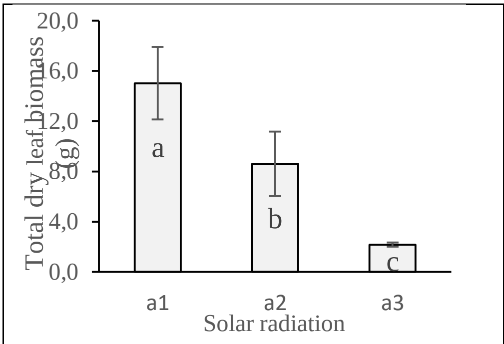

Figure 1. Total dry leaf biomass under different levels of solar radiation

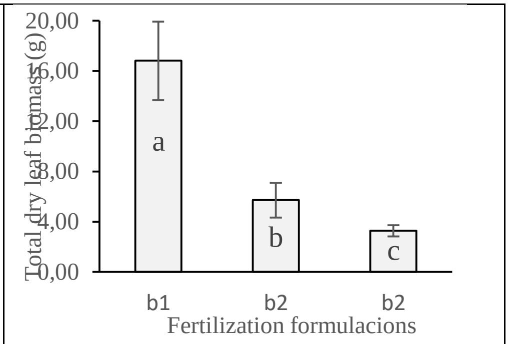

Figure 2. Total dry leaf biomass under different fertilization formulations

Figures 3 and 4 show the behavior of the interaction ($P< 0.05$) between the levels of solar radiation and the different fertilization formulations in the variables total fresh biomass of leaf and total fresh biomass of stem. What is observed is that under 100% solar radiation, the effect of the fertilization formulations is very different on the amount of total biomass that is produced, while at 50% solar radiation, the formulations have an almost similar in biomass production. Exactly the same is true for the production of total fresh stem biomass. This means that the effect of the fertilization formulations on the growth and productivity variables of *Crotalaria longirostrata* will depend on the percentage of solar radiation.

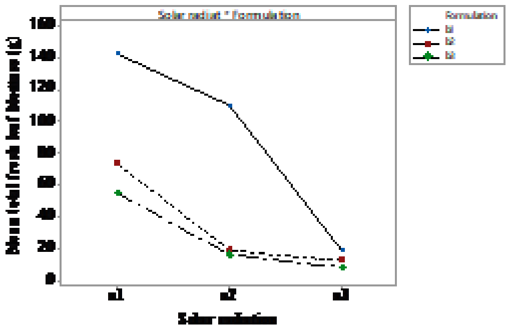

Figure 3. Interaction of solar radiation levels and fertilization formulations for the variable total fresh leaf biomass.

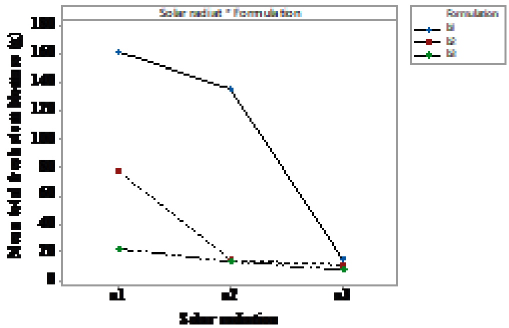

Figure 4. Interaction of solar radiation levels and fertilization formulations for the variable total fresh stem biomass.

In figure 5 a marked linear trend is observed since the dependent variable of fresh total leaf biomass increases as the variable basal stem diameter increases. This is confirmed by the regression equation, where the slope turns out to be 1.813, demonstrating that the basal stem diameter is a variable directly correlated with the biomass production of the aerial part of the *Crotalaria longirostrata* plant. The validation of the model is significant with $r^2 = 0.98$, which indicates an explained variation rate of 98%. This result will allow estimating the biomass productivity of the plant, knowing the basal diameter of the stem, without having to destroy the plant.

$$\hat{Y} = -7.422 + 1.813X \qquad r^2 = 0.98$$

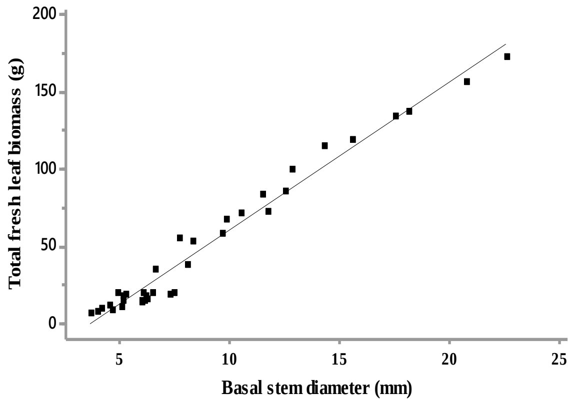

Figure 5. Linear regression line of the total fresh leaf biomass as a function of the basal stem diameter of *Crotalaria longirostrata.*

CONCLUSIONS

The *Crotalaria longirostrata* plant exposed to 100% solar radiation and a fertilization formula containing the three macronutrients turned out to be the best conditions for a greater production of dry and fresh biomass in leaf, stem and root. Similarly for the basal stem diameter and number of lateral branches. The amount of fresh leaf biomass depends on the basal stem diameter.

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If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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