



RESPONSE OF SOME COWPEA VARIETY (Vigna unguiculata) TO THE ALLELOPHATIC EFFECT OF Khaya senegalensis AND Eucalyptus camaldulensis

^{1*}MOHAMMED, U. A., ¹DANJUMA, O., ²SULEIMAN, K., ³AISHA, B. M. AND ³SAFWAN, I. I.¹

¹Department of Biological Sciences, Bauchi State University, Gadau. Bauchi State, Nigeria. P.M.B 65

²Department of Biological Sciences Bayero University, Kano, Nigeria. ³National Biotechnology Development Agency, Federal Ministry of Science and Technology, Abuja, Nigeria.

*Corresponding Author: farukamohammed@gmail.com

ABSTRACT

Availability of land for arable farming is a major concern with increase in population which leads to needs for more infrastructures and more farmers. Some of these fields used have economic trees with allelopathic effects. It is therefore important to understand this allelophatic effects and find a sustainable solution. The allelopathic inhibitory effects of leaf extracts of *Khaya senegalensis* and *Eucalyptus camaldulensis* were investigated on the preliminary growth of three Cowpea (*Vigna unguiculata*) genotypes; Aloka Aloka, IT99K-573-1-1 and IT99K-573-2-1 at 3% and 5%. A randomized complete block design was employed in small plastic bags. Growth parameters such as shoot and root length, number of leaves, stem girth and leaf area index were determined. The study revealed that leaves extracts of *Eucalyptus camaldulensis* and *Khaya senegalensis* significantly (p < 0.05) affected the growth of the three cowpea genotypes, Aloka Aloka, IT99K-573-1-1 and IT99K-573-2-1 with increase in concentration. This clearly indicated the inhibitory properties the leaves extracts possess. The findings in this study indicated that IT99K-573-1-1 tolerated the inhibition more at 3% and 5% compared to the other genotypes. Farmer can plant this cowpea genotype, IT99K-573-1-1 close when they have *Khaya senegalensis* and *Eucalyptus camaldulensis* in or around there crop fields.

Keyword: *Khaya senegalensis* and *Eucalyptus camaldulensis*, Allelophaty, Cowpea, and leaf extract.

INTRODUCTION

Allelophaty is now considered a sub-displine of chemical ecology that deals with the effect of chemical produced by microorganisms or plants on the growth and development of other microorganisms and plants in natural communities or agricultural systems (Einhelling, 1995; Findura, 2020). The term allelopathy is coined from the Greek words allelo and pathy (meaning "mutual harm "or "suffering "). The process or rather the phenomenon of allelopathy in plants occurs when a plant species actively interferes chemically with the germination,

development and growth of other neighboring plant species has been known for years. In a statement as far back as 300 years, indicated that a lot of crop plants such as barley (*Hordeu valgare*) and Chickpea (*Cicer arietinum*) inhibited the growth of weed and crops plant other than barley (Rice, 1984; Kruse *et al.*, 2000). All organs of plants contain allelochemicals

All organs of plants contain allelochemicals they produce or just store them. These chemicals are usually found in different concentration in each location (Gatti *et al.*, 2010). The amount and emission pathway differ ammong species (Friedman 1995;



Monica et al., 2013). The International Allelopathy society (IAS) defines allelopathy in 1996 an adverse influence of one plant or microorganisms on another (Rice, 1984). Allelopathy can be used intentionally for control of weed in agriculture (Kohli et al., 1998). It is also documented; allelopathy may play the most important role in plant interference by those chemical compounds (Inderjit and Dakshin, 1992). Most of those compounds are released in to environment through leaching, litter decomposition, direct volatilization or by a root exudation, and affect (positive negatively) could or germination and growth of other species (Gross and Parthier 1994; Seligler 1996).

Several plants parts such as rots, stems, leaves, rhizomes, seeds, pollen and flowers can contain allelochemicals. These allelochemical have the ability to affect the physiological functions of plants such as germination, photosynthesis, respiration, ion uptake, water status, enzymes activities and transportation. It can also affect the hormones level, stomatal function, cell division and differentiation, gene expressions and cell membrane and wall structure (Chon and Nelson 2010; Zhou and Yu 2006; Reigosa et al., 1999; Monica et al., 2013). Allelochemicals can also influence neighbouring species indirectly by modifying abiotic and biotic interactions, like nutrient uptake and bioavailability or affecting microorganism population in the soil (the effect can always be either positive or negative) (Tharayil, 2009; Fernandez et al., 2013). However, the concentration of these allelochemical may either affect plants negatively when the concentration is high or positive negatively when the concentration is low and this is known as hormesis (Hadacek et al., 2011; Monica et al., (2013).

Cowpea (*Vigna unguiculata*) is one of the most important legume crop that is widely grown with a low input system of production and in semi-arid and arid agro-ecologies on

earth. It is mostly made up of a self-fertilizing crop with grains that have a high amount of protein (19 - 35%) that is rich with two important essential amino acids, namely tryptophan and lysine (Ibro et al., 2014; Abadassi, 2015; Lydia and Shimelis, 2020). Cowpea is also known with many names that includes black eye pea, southern pea, crowder pea, lubia and coupe or frijole. Cowpea is a member of the family Fabaceae and the subfamily Faboideae (Agbogidi, 2010). Nigeria is the leading producer of cowpea grains with an annual approximate production of 2.14 tonnes. million metric Other leading producers include USA, Peru, Serbia, Sri Lanka, China (FAOSTAT, 2017). There is an urgent need to increase the production of cowpea (Vigna unguiculata) in Nigeria due to the ever increase in population, since that, the yield product of cowpea in Nigeria is very low, due to some causing effect, while the population is rapidly increasing every day (Lydia and Shimelis, 2020).

MATERIALS AND METHODS

Study Area and Seeds Collection

The study was carried out at botanical garden Bauchi State University Gadau and the leave extract preparation was also carried out in the laboratory of the Bauchi State University Gadau; situated between Lat.11.50° to 11.83° N and Long. 10.10° to 10.16° E. Bauchi State occupies a total land area of 49,119 km 2 (18,965 sq mi) representing about 5.3% of Nigeria's total land mass and is located between latitudes 9° 3' and 12° 3' north and longitudes 8° 50' and 11° east The state is bordered by seven states, Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east and Kaduna to the west. Bauchi State is one of the states in the northern part of Nigeria that span two distinctive vegetation zones, namely, the Sudan savannah and the Sahel savannah. Seeds of improved cowpea (Vigna





unguiculata L.) genotypes were purchased from Bauchi State Agricultural Development program (BSADP).

Preparation of Aqueous Extracts

The preparation of the aqueous extracts of Khaya senegalansis and *Eucalyptus* camaldulensis was carried out as described by Jafari et al., (2007). Fresh leaves of each senegalansis and *Eucalvptus* Khava species camaldulensis were collected separately. For each species, 250g of the fresh leaves were shade dried at room temperature and finely ground with a mortar and pestle. The ground plant material was soaked in 2 L of water for 12 hours. The solution was filtered through cheese cloth to remove debris and the filtrate was further filtered through Whatman No. 1 filter paper. The final extract solution, which served as stock (100%) was diluted appropriately with water to give 3 and 5% concentrations of the aqueous extracts: while distilled water was used as the control. The two dilutions of extract from Khaya senegalansis and Eucalyptus camaldulensis leaves were considered as treatments used in this experiment. The filtrates were kept in the refrigerator for 7 days to maintain its freshness and to prevent degradation of its allelochemicals before use.

The treatment were arrange in completely randomized design with three replications. Parameters recorded were shoot length, root length, number of leaves and leaf area index. 10g fresh leaves of these two plants were homogenized in1000cm3 distilled water for 24h (one day). A 5% and 3% concentration of the leaves extract were made and store separately in conical flasks. Distill water were use as control (0%).

Experimental layout for Allelophatic Effect of *Khaya senegalansis* and *Eucalyptus camaldulensis* on *Vigna unguiculata L*. Genotypes

Cow manure is use for soil mixture in an accurate ratio to that of sand, using hand gloves and difference genotypes seed of cowpea were used in this research which are IT99K-573-1-1, IT99K-573-2-1 and Aloka. 54 plastic pots were used in the experiment. The pots were placed in the botanical garden of Bauchi State University Gadau main campus and arranged in a complete randomised design. The experiment consist of two different concentration of aqueous extract of two plants, which are *Khaya senegalansis* and *Eucalyptus camaldulensis* extracted at 3% and 5% and a control (distilled water).

Treatments

The following treatments were use in three different cowpea varieties (*Vigna unguiculata*) in the experiment.

T0 = Seeds of receptor plants grown in 0% (control)

T1 = Seeds of receptor plants grown in leaf extracts of 3% concentrations of *Khaya senegalansis* and *Eucalyptus camaldulensis* leave extract

T2 = Seeds of receptor plants grown in leaf extracts of 5% concentrations *Khaya* senegalansis and *Eucalyptus camaldulensis*.

Data Analysis

The data will be subject to analysis of variance (ANOVA) and where there is significant difference the means will be separated appropriately.



RESULTS AND DISCUSSION

Allelophatic Effect of Effect of Khaya senegalansis and Eucalyptus camaldulensis on Growth Parameters of Vigna unguiculata L. Genotypes

The mean shoot length, root length, number of leaves, stem girth and leaf area index of the treatment and varieties were analysed and compared in table 1. The control recorded the highest mean shoot length among the treatments with the value 7.688 followed by 5.299 cm recorded by Khaya senegalensis (3%), while Eucalyptus camaldulensis (5%) recorded the least with the value 4.958 the differences were significant at p < 0.05. The cowpea variety that recorded the highest mean shoot length was IT99K-573-1-1 which recorded 6.377 cm while IT99K-573-2-1 with the value 4.869 cm recorded the least the difference were also significant at p < 0.05. In terms of root length the control recorded the highest value (5.979 cm) among the treatments while Eucalyptus camaldulensis (5%) recorded the least with the value 3.961 cm and these values are significantly different (p > 0.05). On the other hand IT99K-573-1-1 with the value 5.161 cm had the highest mean root length among the cowpea genotypes while Aloka Aloka had the least (4.375 cm) which was significantly different at p > 0.05.

Similarly, the control was observed to have more number of leaves (10.222) among the treatments while *Eucalyptus camaldulensis* (5%) had the least (5.0) and these difference were significant at p > 0.05. The cowpea genotypes did not differ significantly (p > 0.05) where IT99K-573-1-1 was observed to have recorded the highest mean number of leaves (7.0) while the least value 6.333 was recorded by IT99K-573-2-1. The stem girth mean values among the treatments and cowpea genotypes differ significantly (p > 0.05) and the control also recorded the highest with 1.123 cm while *Eucalyptus camaldulensis* (5%) had the least with the value 0.358 cm. Aloka Aloka recorded the the highest mean stem girth with the value 0.716 cm while the least was observed on IT99K-573-2-1 with the value 0.485 cm. The mean leaf area index observed in cm among both the treatments and cowpea genotypes were significantly different at p > 0.05. Similarly, the control has the highest value (11.67) while *Khaya senegalensis* 5% had the least (2.541) among the treatments. On the other hand, IT99K-573-1-1 with the value 6.745 while the least

was observed on IT99K-573-2-1 with the

value 3.475. The means of the interaction between the leave extracts and the cowpea genotypes as 2 presented in Table indicated that Khavasenegalensis 3% vs IT99K-573-1-1 and Eucalyptus 3% vs IT99K-573-1-1 recorded the best shoot length with the values 6.390 cm and 6.197 cm respectively. While the interaction between Eucalyptus 5% vs IT99K-573-2-1 recorded the least shoot length (4.107 cm). The best interaction with the root length was between Khayasenegalensis 3% vs IT99K-573-1-1 and Khayasenegalensis 3% vs IT99K-573-2-1 with the values 5.467 cm and 5.367 cm respectively. The least was recorded by Eucalyptus 5% vs Aloka (3.183 cm). Khayasenegalensis 3% vs IT99K-573-1-1 recorded the highest number of leaves with the value 7.667 while Eucalyptus 3% vs IT99K-573-2-1 and Eucalyptus 5% vs Aloka had the least with the values 4.333 and 4.333 respectively. The effect of the interactions on the stem girth revealed Eucalyptus 3% vs IT99K-573-1-1 had the best value (0.920 cm) compared to the others while Khayasenegalensis 5% vs IT99K-573-2-1 recorded the least (0.340 cm). Eucalyptus 3% vs IT99K-573-1-1 recorded the highest leaf index (7.590)area cm) while Khayasenegalensis 5% vs IT99K-573-2-1 recorded the least (2.140 cm).



The leaf extracts of the two plant species senegalansis *Eucalyptus* Khava and camaldulensis used significantly affected the growth of the cowpea genotypes used in this research at p < 0.05 when compared to the control in Table 1. The inhibition of the three cowpea genotypes, namely Aloka Aloka, IT99K-573-1-1 and IT99K-573-2-1observed could be attributed to a contribution of allelochemicals present in the leaf extracts of senegalansis and Khaya Eucalyptus camaldulensis. A lot of research work have indicated that the allelochemicals are watersoluble and have the potential to accumulate upon release within the seeds in direct contact with bioactive concentrations (Fara et al., 2014). The result in this study agreed with the findings of (Hayatu et at., 2016) who observed the effect of Eucalyptus camaldulensis on cowpea seeds and reported inhibition.

In this study, the interaction between the leaf extracts and cowpea genotypes used indicated inhibition of growth using the indices of growth like shoot and root length, number of leaves, stem girth and leaf area index.. This is in line with the findings of (Sasikumar *et al.*, 2004; Popoola *et al.*, 2020); they reported that the extracts from leaves of *Eucalptus sp.*, *Chromolaena odorata*, *Euphorbia heterophylla* and *Tridax procumbens* are common plants with inhibitory effects on the growth of radicle and plumule of cowpea (Vigna unguiculata).

The cowpea genotype IT99K-573-1lperformed better in both leaves extracts compared to the other genotypes Aloka Aloka and IT99K-573-2-1, which indicated it possess the attributes to withstand the allelopathic effects of the leave extracts.

Table 1: Means of shoot length, root length, number of leaves, stem girth and leaf area index in relation some cowpea genotypes subjected some plants extracts.

	Shoot length (cm)		Root length (cm)		Number of leaves		Stem girth (cm)		Leaf area index (cm)	
Treatment	Mean	SE (±)	Mean	SE (±)			Mean	/	Mean	SE (±)
Control	7.688ª	0.06	5.979ª	0.045	10.222ª	()	1.123ª		11.670ª	0.129
Eucalyptus 3%	5.287 ^b	0.06	4.691°	0.045	5.333°	0.302	0.740^{b}	0.043	6.206 ^b	0.129
Eucalyptus 5%	4.958°	0.06	3.961°	0.045	5.000°	0.302	0.358°	0.043	2.714 ^d	0.129
Khaya senegalensis 3%	5.299 ^b	0.06	5.394 ^b	0.045	6.778 ^b	0.302	0.463°	0.043	3.951°	0.129
Khaya senegalensis 5%	4.978°	0.06	4.456 ^d	0.045	5.778 ^{bc}	0.302	0.363°	0.043	2.541 ^d	0.129
Varieties										
Aloka Aloka	5.679 ^b	0.049	4.375 ^b	0.035	6.533ª	0.234	0.716 ^a	0.033	6.030 ^b	0.100
IT99K-573-1-1	6.377ª	0.049	5.161ª	0.035	7.000^{a}	0.234	0.628 ^a	0.033	6.745 ^a	0.100
IT99K-573-2-1	4.869°	0.049	5.153ª	0.035	6.333ª	0.234	0.485 ^b	0.033	3.475°	0.100

Values refer to means and standard error. Means that do not share the same letter are significantly different at 95% confidence level.





Table 2: Means of the interaction between leave extracts and cowpea genotypes for shoot length, root length, number of leaves, stem girth and leaf area index in relation some cowpea genotypes subjected some plants extracts.

Leave extract* genotypes Shoot le		ength Root length		Number of		Stem girth		Leaf area index		
					leaves					
	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)	Mean	SE (±)
Controlvs Aloka	7.196 ^b	0.109	5.600 ^b	0.079	10.333 ^{ab}	0.523	1.003 ^b	0.074	9.363 ^b	0.223
Control vs IT99K-573-1-1	8.467 ^a	0.109	6.140 ^a	0.079	10.667 ^a	0.523	1.433 ^a	0.074	20.140 ^a	0.223
Control vs IT99K-573-2-1	7.400 ^b	0.109	6.197ª	0.079	9.667 ^{ab}	0.523	0.933 ^b	0.074	5.507 ^d	0.223
Eucalyptus 3% vs Aloka	5.197 ^d	0.109	3.240 ^d	0.079	5.333 ^{cd}	0.523	0.897 ^b	0.074	7.357°	0.223
Eucalyptus 3% vs IT99K-573-1-1	6.197°	0.109	5.333 ^b	0.079	6.333 ^{cd}	0.523	0.920 ^b	0.074	7.590°	0.223
Eucalyptus 3% vs IT99K-573-2-1	4.467°	0.109	5.500 ^b	0.079	4.333 ^d	0.523	0.403°	0.074	3.670 ^e	0.223
Eucalyptus 5% vs Aloka	5.300 ^d	0.109	3.183 ^d	0.079	4.333 ^d	0.523	0.387°	0.074	3.143 ^{ef}	0.223
Eucalyptus 5% vs IT99K-573-1-1	5.467 ^d	0.109	4.433°	0.079	5.333 ^{cd}	0.523	0.343°	0.074	2.760 ^{ef}	0.223
Eucalyptus 5% vs IT99K-573-2-1	4.107 ^e	0.109	4.267°	0.079	5.333 ^{cd}	0.523	0.343°	0.074	2.240^{f}	0.223
Khayasenegalensis 3% vs Aloka	5.203 ^d	0.109	5.350 ^b	0.079	6.000 ^{cd}	0.523	0.890^{b}	0.074	7.260°	0.223
Khayasenegalensis 3% vs IT99K- 573-1-1	6.390°	0.109	5.467 ^b	0.079	7.667 ^{bc}	0.523	0.095°	0.074	0.777 ^g	0.223
Khayasenegalensis 3% vs IT99K- 573-2-1	4.303 ^e	0.109	5.367 ^b	0.079	6.667 ^{cd}	0.523	0.403°	0.074	3.817°	0.223
Khayasenegalensis 5% vs Aloka	5.500 ^d	0.109	4.500°	0.079	6.667 ^{cd}	0.523	0.403°	0.074	3.027 ^{ef}	0.223
Khayasenegalensis 5% vs IT99K- 573-1-1	5.367 ^d	0.109	4.433°	0.079	5.000 ^{cd}	0.523	0.347°	0.074	2.457 ^f	0.223
Khayasenegalensis 5% vs IT99K- 573-2-1	4.067 ^e	0.109	4.433°	0.079	5.667 ^{cd}	0.523	0.340°	0.074	2.140 ^f	0.223

Values refer to means and standard error. Means that do not share the same letter are significantly different at 95% confidence level.

CONCLUSION

The study indicated that leaves extract of *Eucalyptus camaldulensis* and *Khaya senegalansis* affected the growth of the three cowpea genotypes, Aloka Aloka, IT99K-573-1-1 and IT99K-573-2-1 with increase in concentration because they possess inhibitory properties. Although affected IT99K-573-1-1 performed better than the rest of the cowpea genotypes used in the study

REFERENCES

- Abadassi, J. (2015). Cowpea (Vigna unguiculata (L.) Walp.) agronomic traits needed in tropical zone Int. J. Pure app. Biosci., 3 (2015), pp. 158-165.
- Agbogidi, O. (2010). Screening six cultivars of cowpea (Vignia unguiculata L.) walp for adaptation to soil contaminated with spent engine oil. J. Environ. Chem. Ecotoxicol., 2 (2010), pp. 103-109.



- Chon, S. U. and Nelson, C. J. (2010). Allelopathy in compositae plants. A review. *Agron. Sustain. Dev.* 30, 349–358.
- Einhellig F. A. (1995). "Allelopathy-current status and future goals," in *Allelopathy:* Organisms, Processes, and Applications, eds Inderjit A., Dakshini K. M. M., Einhellig F. A. (Washington, DC: American Chemical Society Press;), 1–24.
- FAOSTAT, (2017). Available online: http://www.fao.org/faostat/en/#data.Q <u>C</u> (2017) (accessed on January 2018).
- Fara WA, Hamidi AMI, Fahmi HI, Zainuddin MYH (2014). Preliminary Study on Allelopathic Effect from Chromolaena odorata (Siam Weed) Leaves Extracts Towards Vigna radiate. International Journal of Engineering Research and Technology 3:8.
- Fernandez, C., Santonja, M., Gros, R., Monnier, Y., Chomel, M. & Baldy, V. (2013). Allelochemicals of *Pinus halepensis* as drivers of biodiversity in Mediterranean open mosaic habitats during the colonization stage of secondary succession. *J. Chem. Ecol.* 39, 298–311.
- Friedman, J. (1995). Allelopathy, autotoxicity, and germination. pp. 629-644. In: J. Kigel; G. Galili. (eds.). Seed Development and Germination. New York, Marcel Dekker.
- Gatti, A B., Ferreira, A. G., Arduin, M., Cristina, S. & Perez, G. A. (2010). Allelopathic effects of aqueous extracts of Artistolochia esperanzae O.Kuntze on development of Sesamum indicum L. seedlings. *Acta Bot. Bras.* 24 (2).
- Gross, D. and Parthier, B. (1994). Response to allelopathy. *Journal Plant Growth Regulation*. 13: 93-114.
- Hadacek, F., Bachmann, G., Engelmeier, D. & Chobot, V. (2011). Hormesis and a

chemical raison d'être for secondary plant metabolites. *Dose-Response.* (2011); 9(1):79–116.

- Hayatu, M., Mohammed, U. A. and Juji, H.A. (2016). Effect of *Moringa oleifera* and *Eucalyptus camaldulensis* Leaf Extracts on Germination and Growth of Some Local and Improved Cowpea(*Vigna unguiculata* L. Walp) Genotypes. *Best Journal* 13(2): 140 – 145.
- Ibro, G., Sorgho, M. C., Idris, A. A., Moussa, B., Baributsa, D. & Lowenberg-DeBoer, J. (2014).
 Adoption of cowpea hermetic storage by women in Nigeria, Niger and Burkina Faso. J. Stored Prod. Res., 58 (2014), pp. 87-96.
- Inderjit, K. & Dakshini, M. M. (1992). Interference potential of Pluchea lanceolata (Asteraceae): Growth and physiological responses of asparagus bean *Vigna unguiculata var.* sesquipedalis. Amer. J. Bot. 79: 977-981.
- Jafari, L., Kholdebarin, B. and Jafari, E. (2007). Phytotoxic Effects of Chenopodium album L. Water Extract on Higher Plants. American Journal of Plant Physiology, 2: 221-226.
- Kohli, R.K. Batish, D & Singh, H.P. 1998. Allelopathy and its implications in agro-ecosystems. Journal of Crop Production, 1: 169-202.
- Kruse M, Strandberg M, Strandberg B. 2000. Ecological Effects of Allelopathic Plants- A Review. NERI Technical Report, No. 315, Ministry of Environment and Energy National Environmental Research Institute.
- Lydia, N. H., & Shimelis, H. (2020). Production constraints and breeding approaches for cowpea improvement for drought prone agro-ecologies in Sub-Saharan Africa. *Annals of*



Agricultural Sciences. (2020) 65(1): 83-91.

- Mallik A. U. (2003). Conifer regeneration problems in boreal and temperate forests with ericaceous understory: Role of disturbance, seedbed limitation, and keystone species change. *Crit. Rev. Plant Sci.* 22, 341– 366.
- Monica, B. (2013). Allelopathic effect of Festuca rubra on perennial grasses. *Romanian Biotechnological Letters* 18(1): 8190 -8196.
- Popoola, K. M., Akinwale, R. O. and Adelusi, A. A. (2020). Allelopathic effect of extracts from selected weeds on germination and seedling growth of cowpea (Vigna unguiculata (L.) Walp.) varieties. African Journal o Plant Science. Vol.14(9) 338-349.
- Reigosa, M.J., Sánchez Moreiras, A.M. & González, L. 1999. Ecophysiological approach in allelopathy. *Critical Reviews in Plant Sciences* 18: 577-608.
- Rice E. L. (1984). *Allelopathy*, 2nd ed. New York: Academic Press.
- Seligler, D. S. (1996). Chemistry and Mechanism of Allelopathic Interactions. Agronomy Journal, 88, 876-885.
- Zhou, Y.H. & Yu, J.Q. (2006).
 Allelochemicals and photosynthesis, pp. 127–139. In: Reigosa M.J., Pedrol N. & González L. (eds), Allelopathy. Springer Netherlands