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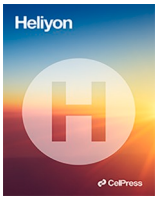

Research article

Increasing the planting density of *Cryptolepis sanguinolenta* (Lindl.) Schlt increased root biomass and cryptolepine yield

Jacqueline Naalamle Amissah [a,*], Frank Opoku-Agyemang [a], Freda Elikplim Asem [b], Dorcas Osei-Safo [c], Ivan Addae-Mensah [c]

[a] *Department of Crop Science, University of Ghana, P. O. Box LG 44, Legon, Accra, Ghana*
[b] *Department of Agricultural Economics and Agribusiness, University of Ghana, P.O. Box LG 68, Legon, Accra, Ghana*
[c] *Department of Chemistry, University of Ghana, P. O. Box LG 56, Legon, Accra, Ghana*

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ABSTRACT

Cryptolepis sanguinolenta (Lindl.) Schlt. is an important multipurpose medicinal plant used for the treatment of ailments such as malaria. Despite the ongoing efforts in domesticating the herb, the ideal planting density and its benefits are unknown. A study was conducted to determine the influence of six *C. sanguinolenta* accessions and three planting densities (15, 30 and 45 plants/1.8 m^2) on root biomass, cryptolepine concentration and cryptolepine yield. Also, benefit-cost ratios were determined for each plant density across the four cultivation periods (9, 12, 15 and 18 months). The cultivation of *C. sanguinolenta* at the highest planting density (45 plants/1.8 m^2) increased root biomass (value), cryptolepine content (2.08 mg/100 mg dry root) and cryptolepine yield (23.31 mg mg/1.8 m^2) compared to those cultivated at lower planting densities (15 and 30 plants/1.8 m^2). The duration for growing *C. sanguinolenta* had a more significant influence on cryptolepine yield but not the cryptolepine content. Plants cultivated for 15 months gave the maximum cryptolepine yield (10.33 g/bed), indicating 15 months as the optimum time to harvest the roots. The benefit-cost analysis revealed that growing the plant at a density of 45 plants/1.8 m^2 (25,920 plants/acre) for 18 months was a more profitable venture with a benefit-cost ratio of 3.45. Commercial cultivation of *C. sanguinolenta* at 45 plants per bed area of 1.8 m^2 (25,920 plants/acre) for 15–18 months is recommended as the most profitable and promising cropping practice to ensure the sustainable supply of planting material.

1. Introduction

Cryptolepis sanguinolenta (Lindl.) Schlt, belonging to the Apocynaceae family and Periplocaceae sub-family, is important in West African ethnomedicine due to its anti-inflammatory, anti-hypertensive, antithrombotic, antidiabetic, antiplasmodial, and antipyretic properties [1]. It is native to West Africa and in Ghana, it is widely distributed in areas with adequate rainfall such as the Akwapim and Aburi mountains [2]. *C. sanguinolenta* has been used for the treatment of malaria in Ghana for generations [3]. In addition to its main constituent alkaloid, cryptolepine, several other similar alkaloids have been isolated from the plant [4]. It is included in several plant-based products being sold on the market in Ghana [5]. The plant is locally known as nibima, kadze, gangamau, and yellow dye

* Corresponding author.
E-mail addresses: jnamissah@ug.edu.gh (J.N. Amissah), frankopoku35@gmail.com (F. Opoku-Agyemang), fasem@ug.edu.gh (F.E. Asem), dosei-safo@ug.edu.gh (D. Osei-Safo), iaddae-mensah@ug.edu.gh (I. Addae-Mensah).

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with its root system being the most essential part of the plant in that it contains about 90 % of the indoloquinoline alkaloids present in the plant with cryptolepine accounting for the bulk of the antimalarial activity in its crude extracts [6,7].

In addition to its use in treating malaria, *C. sanguinolenta* and its constituents have been found to possess antimycobacterial [8], antimicrobial [9,10], antihyperglycemic [11], and anticancer [12] potential. According to Opoku-Agyemang et al. [13] it is used to treat diseases such as Babesia, Lyme disease (*Borreliosis burgdorferi*) and Bartonella in the United States of America. In January 2021, the Food and Drugs Authority (FDA) of Ghana approved the plant for clinical trials as a potential treatment for the Coronavirus disease (COVID-19) [14]. According to Amissah et al. [15], the current medicinal plant industry in Ghana is not only for satisfying health care needs but also a source of wealth-creation for plant material collectors and plant medicine manufacturing companies, whose collective activities contribute to the economic growth of the country. , *C. sanguinolenta* is currently not cultivated as an industrial crop. It is collected from the wild, and the demand for its roots and its widespread use have resulted in the destructive harvesting of the plant. This non-sustainable practice has already caused a substantial depletion of wild populations in Ghana and the destruction of entire populations, resulting in habitat loss in parts of its native distribution [15–17]. Such human activities also negatively impact biodiversity at gene, species, community and ecosystem levels resulting in habitat fragmentation, eroding of natural and adaptive genetic diversity, reduce effective population size, lower evolutionary potential and ultimately species extinction [18,19]. The current situation calls for immediate sustainable management of the plant.

Just as is the case with several important medicinal plants, there is very little effort in the conservation of *C. sanguinolenta* wild populations. To ensure sustainable *C. sanguinolenta* conservation and cultivation, identification of high-performing adaptable cultivars, the institution of appropriate agronomic practices such as optimum plant density, which are easy to adopt, and determination of the cryptolepine concentration (principal active ingredient) are highly recommended [15,20,21]. These measures will ensure a reliable and steady supply of plant material and increase the profit margin of collectors despite the threats of forest clearings for farming and other activities. Cultivation of *C. sanguinolenta* can result in the identification of superior raw materials harvested at technological maturity, adequately dried, and in properly processed forms for effective active ingredient determination, requirements that cannot be met by wildcrafting [22]. Plant density is one of the agronomic techniques used in achieving optimum yield. According to Mirzaei et al. [21], the choice of a particular plant density is dependent on the plant's growth characteristics and cultivation methods to mention a few. A recommended number of plants per unit area contributes to adequate utilization of the available planting space, thus ensuring an even distribution of water, nutrient, light, and air [23]. A study by Panahandeh et al. [24] showed that plant density significantly improved the length, girth, and root fresh weight of *Cichorium intybus.* Other studies have shown that plant density significantly influences the root yield of *Beta vulgaris* L. and *Manihot esculenta* Crantz [25,26]. Also, profitable commercial cultivation of medicinal plant species such as *C. sanguinolent* must consider the cost of labour, land, and other agricultural inputs [27]. Commercial production of medicinal plants is mostly compromised if wild-harvested plant samples are still available at a lower price. Hence, bringing *C. sanguinolenta* cultivars into cultivation is dependent on the adequate economic calculation of superior cultivars influenced by the price of weightier roots and those with high active ingredient concentration [28].

The study was carried out to evaluate the effect of plant density and accession on root biomass yield and cryptolepine concentration of *C. sanguinolenta*. Also, the relationship between production costs and profits in its cultivation was determined. The study hypothesizes that variations in plant density and accession would have a significant impact on the root biomass yield and cryptolepine concentration of *Cryptolepis sanguinolenta*. Additionally, there would be a significant relationship between production costs and profits in the cultivation of this plant species.

2. Materials and methods

2.1. Field studies

The field study was conducted at a research farm that has been under grass fallow for five years at Aburi in the Eastern Region of Ghana (Latitude: 5.85118360, Longitude: −0.17291060, and Elevation: 461.30 m above sea level) from September 2019 to April 2021. The research area is in the tropical rainforest zone with annual average rainfall and temperature of 1565 mm and 25.9 °C respectively. The soil in the study area was typically Haplic Lixosol belonging to the Kokofu soil series of Ghana as described by the FAO-UNESCO classification [29]. Soil particle analysis revealed a sandy loam soil (73.9 % Sand, 7.5 % Clay and 18.7 % Silt). The pre-planting soil chemical analysis showed that it had Nitrogen 0.23 %; organic Carbon 3.96 %; Available P 10.16 mg kg^{-1}; pH (1:1H_2O) 7.1; Electrical conductivity (1:1H_2O) 134.13; Calcium 5.87 Cmol kg^{-1}; Magnesium 0.96 Cmol kg^{-1}, Potassium 0.52 Cmol kg^{-1}; Sodium 0.85 Cmol kg^{-1} and Cation Exchange Capacity (CEC) 24.90 Cmol kg^{-1}.

2.2. Planting material and establishment

Seeds of *C. sanguinolenta* accessions were obtained from plants previously collected from the wild and established in the Sinna Garden of the Department of Crop Science, University of Ghana under field conditions [30]. Seedlings were raised in nursery bags of dimension (6" × 4") filled with sandy loam soil. Recommended nursery practices were observed to ensure that vigorous and uniform seedlings were obtained. Before transplanting, raised beds of dimensions 3 m × 0.6 m, i.e., an area of 1.8 m^2 were prepared. Eight weeks after sowing, seedlings with an average height of 20 cm with 10–15 matured leaves were selected and transplanted onto the well-prepared beds in the field and spaced at 20 cm × 20 cm, 30 cm × 20 cm and 60 cm × 20 cm. A 6 × 3 factorial treatment arranged in a Randomized Complete Block Design (RCBD) with three replications was used. Two factors; accession and plant density were used, with six accessions (176KAA, 77KAA, 15DNN, 40HO, 96 KG, 201 KA) and three plant densities (15, 30 and 45 plants/1.8 m^2). The

plants of the different accessions at varied plant densities were harvested at different plant ages (9, 12, 15 and 18 months) after planting. Previous studies determined the 9th month to be the optimum time at which cryptolepine concentration is highest in the plant's roots [15,31]. Cultural practices such as watering, weeding and reshaping of beds, were carried out as and when necessary.

2.3. Data collection

Data (fresh shoot and root weights, dry shoot and root weights and moisture content of roots) were collected on all plants per bed per replicate in the first experiment. Harvested plants were separated into shoots and roots and their weights were recorded. Subsequently, shoot and root samples were air-dried for 14 days before dry weights were taken using a digital scale (Leion Engineering, India). The moisture content of the root samples was determined using a Moisture Content Meter (Mettler-Toledo GmbH, SWITZERLAND). The shoot-to-root ratio was computed using Microsoft Excel.

2.4. Soil analysis

Soil macronutrient content analysis was performed using soil samples taken using a soil auger (10 cm barrel and 5 cm diameter) at a depth of 15–25 cm before planting. The samples were thoroughly mixed, and the nutrient analysis was replicated three times, using a fresh sample each time.

2.5. Determination of active ingredient concentration in roots of Cryptolepis sanguinolenta

The cryptolepine concentrations in the roots of the plants were determined at 9, 12, 15 and 18 months for the different plant densities [15, 30 and 45 plants per bed (ppb)]. Root samples collected for the different plant densities were air-dried, ground, and bulked per accession per harvest period. The crude extracts were obtained by maceration of 100 mg of ground root samples in ethanol (50 ml × 24 h x3) followed by concentration to dryness using a rotary evaporator at 40–45^{o}C. After extraction, all concentrated samples were transferred into pre-weighed and labelled vials by dissolving in HPLC-grade ethanol. Sample vials were left open to evaporate the ethanol. Dried samples were weighed and stored in the freezer (0^{o}C) until HPLC analysis.

2.5.1. High-performance liquid chromatography (HPLC) assay for cryptolepine

The cryptolepine content in the extract was analyzed by HPLC at the Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon, using the Reverse-phase HPLC system on Agilent 1290 Infinity II Preparative LC System; fitted with an Eclipse XDB-C18 150 mm × 4.6 mm; 5 μm column. The mobile phase used was methanol and a water-chloroacetic acid solution at a ratio of 9:1. The water-chloroacetic acid solution was prepared by dissolving 0.7 g of chloroacetic acid in 500 mL of water. The measured pH of the acid solution was 2.42. To ensure that no undissolved particles were injected on the column, solutions of the analyte were prepared up to 1 mL, centrifuged at 13,000 rpm for 2 min, and carefully decanted into clean sample tubes before injection. A calibration curve was determined by dissolving cryptolepine standard (Sigma-Aldrich Corp., St. Louis, MO, USA) in absolute ethanol to prepare solutions of concentrations: 0.1 μg/μL, 0.15 μg/μL, 0.2 μg/μL, 0.5 μg/μL, 1.0 μg/μL, 1.5 μg/μL, 2.0 μg/μL. These standard solutions were injected on the column, run for 15 min at a flow rate of 1 ml/min and a calibration curve was generated from an Agilent OpenLAB CDS ChemStation program (version 2.3.53). One milliliter of each crude extract was placed in vials, loaded onto the autosampler of the HPLC machine after attaining room temperature and run for 15 min at a flow rate of 1 ml/min. Three runs were done and assessed for the precision of the analysis. Chromatograms showing retention time, and intensity of UV absorption were generated from an Agilent OpenLAB CDS ChemStation program (version 2.3.53). These parameters for each sample together with the standard curve were used to compute the cryptolepine concentration in the extract. The UV absorbance of the samples was measured at 366 nm, 254 nm, and 200 nm using an Agilent 1260 Infinity II Diode Array Detector WR.

The economic yield was calculated according to the equation:

Cryptolepine yield (g bed^{-1}) = Dry root biomass (g bed^{-1}) × Cryptolepine content (mg/100 mg of ground dry roots) × 1000 (mg/g) [31]

2.6. Benefit-cost analysis

Material inputs for land preparation (ploughing, harrowing, bed preparation), labour for transplanting of seedlings, harvesting of *C sanguinolenta* roots, fertilizer and nursery bags were recorded. The input value was calculated according to the temporal price of the items and labour per day, while the output value of the dry *C. sanguinolenta* roots was calculated according to the current average market price and computed using the benefit-cost ratio (BCR) of the *C. sanguinolenta* production. Both the input and output values were presented in Ghana cedis (GHC). The BCR was interpreted as; for BCR greater than 1, Net Profit Value (NPV) is greater than the production costs. For BCR equal to 1, the NPV equals the production costs and for BCR less than 1, the NPV is less than the production costs [32–34]. The benefit-cost ratio (BCR) is given as:

$$BCR = \sum B / \sum C$$

Where $\sum B$ = Total net revenue.

$\sum C$ = Total cost of production.

2.7. Statistical analysis

The data collected were subjected to analysis of variance using GenStat statistical software version 19. When the F-test indicated statistical significance at $P = 0.05$, the least significant difference (LSD) at 5 % was used in separating the means.

3. Results and discussion

3.1. Effect of accession, plant density and growth periods on biomass yield of C. sanguinolenta

Accession and plant density significantly affected the dry root and shoot biomass of *Cryptolepis sanguinolenta* from 9 to 18 months after transplanting (MAT) (Tables 1 and 2). Overall, the number of months significantly influenced the effects of the specific interactions on root and the aboveground biomass (Table 2), such that cultivating an accession such as 40HO for 12 and 15 months respectively, at 45 plants per bed (1.8 m^2) increased the aboveground and root biomass compared to the other growth periods. These interaction trends were not consistent throughout the production periods. For example, at 9 MAT, the interaction effects of accession 96 KG at 45 plants per bed (ppb) gave the maximum dry root biomass (0.38 kg) whereas, at 15 MAT, the maximum dry root biomass was obtained when accession 40HO was planted at 45 ppb (1.11 kg) (Table 1). At 18 MAT, the interaction effect of accession 201 KA at a planting density of 45 ppb recorded the highest dry shoot weight (2.03 kg) as compared to accession 176KAA at a planting density of 15 ppb which gave the lowest dry weight (0.32 kg) (Table 2).

Generally, the growth and development of *C. sanguinolenta* at a high plant density was better than at a low plant density. It was evident that plants grown under high plant density produced more biomass which resulted in a high dry shoot (aboveground) and root (below ground) biomass. The study showed that the root biomass (the most economic part of the plant), of *C. sanguinolenta,* increased under high plant density. The increased number of plants per unit area in high plant density provides a dense canopy that maintains sufficient soil moisture for plant uptake, especially under rainfed conditions [35], similar to that in the present study. This ensures the uptake of plant nutrients and essential minerals for improved growth and development [35]. An increase in dry shoot and root biomass production in high plant density has been reported in *Trigonella foenum-graecum* by Singh, Buttar [36]. Such improved biomass production as achieved with high plant density is consistent with previous reports in *Moringa oleifera* [37] and *Fagus orientalis* [38].

The analysis of the dry shoot-to-root ratio, an important indicator of the plant's growth strategy and resource allocation, was significantly influenced by the effects of the specific interactions between accessions and plant density. The ratio of the amount of biomass in the shoots to that in the roots was significantly higher at 18 MAT in the interaction effect of accession 15DNN at a planting

Table 1
Effect of accession and plant density on dry root biomass (g) of *Cryptolepis sanguinolenta*.

Accession	9 MAT	12 MAT	15 MAT	18 MAT
77KAA	199.3 ± 34.1 b	396.0 ± 22.3 ab	509.1 ± 54.4 a	301.0 ± 43.1 a
176KAA	104.3 ± 32.3 a	444.9 ± 20.0 b	620.1 ± 92.0 abc	229.7 ± 57.0 a
15DNN	271.7 ± 18.1 c	501.4 ± 50.0 c	554.1 ± 100.3 ab	511.8 ± 82.7 b
40HO	240.6 ± 42.4c	519.9 ± 89.0 c	719.6 ± 109.0 c	622.4 ± 105.0 c
96 KG	261.0 ± 31.4 c	355.0 ± 54.7 a	652.2 ± 90.4 bc	665.9 ± 36.7 c
201 KA	246.7 ± 29.4 c	387.2 ± 37.4 a	608.3 ± 87.8 abc	705.8 ± 110.8 c
Plant density (plants/bed)				
15 ppb	153.1 ± 22.1 a	385.6 ± 30.6 a	397.7 ± 35.5 a	353.7 ± 41.4 a
30 ppb	225.8 ± 26.6 b	465.3 ± 31.7 b	774.0 ± 48.5 c	425.9 ± 58.8 b
45 ppb	283.0 ± 18.0 c	451.4 ± 46.8 b	660.0 ± 66.2 b	738.7 ± 65.7 c
Accession * Plant density				
77KAA * 15 ppb	115.5 ± 8.4 bc	313.3 ± 13.6 a	604.2 ± 105.3 e	301.7 ± 33.8 bcd
176KAA * 15 ppb	34.3 ± 4.4 a	447.3 ± 16.5 b	335.5 ± 50.3 abc	271.3 ± 130.4 bc
15DNN * 15 ppb	273.7 ± 54.4 gh	553.7 ± 16.6 cd	259.3 ± 33.8 a	296.7 ± 37.4 bcd
40HO * 15 ppb	78.5 ± 6.2 ab	481.3 ± 47.6 bc	498.7 ± 71.7 bcde	291.4 ± 100.7 bcd
96 KG * 15 ppb	197.8 ± 29.7 def	247.3 ± 16.5 a	312.5 ± 46.5 ab	628.8 ± 88.2 fg
201 KA * 15 ppb	218.7 ± 0.4 efg	270.3 ± 71.9 a	376.0 ± 21.4 abcd	332.3 ± 61.1 cd
77KAA * 30 ppb	148.7 ± 0.4 cd	448.3 ± 16.5 b	580.0 ± 57.7 e	159.5 ± 8.6 ab
176KAA * 30 ppb	45.7 ± 2.6 a	424.0 ± 62.4 b	929.0 ± 57.7 fg	69.0 ± 5.9 a
15DNN * 30 ppb	271.5 ± 29.2 gh	639.3 ± 16.5 d	878.0 ± 138.6 f	407.1 ± 22.0 cd
40HO * 30 ppb	325.5 ± 30.3 hij	244.3 ± 0.9 a	551.8 ± 71.4 de	599.3 ± 13.1ef
96 KG * 30 ppb	203.5 ± 2.0 def	571.3 ± 16.5 d	803.0 ± 57.4 f	608.3 ± 14.9 fg
201 KA * 30 ppb	359.7 ± 0.4 ij	464.3 ± 16.5 b	902.0 ± 127.1 f	712.0 ± 57.9 fgh
77KAA * 45 ppb	333.8 ± 7.8 ij	426.3 ± 16.5 b	343.0 ± 15.0 abc	348.8 ± 34.0 cd
176KAA * 45 ppb	233 ± 6.3 fg	463.3 ± 16.5 b	595.8 ± 84.5 e	441.9 ± 33.9 de
15DNN * 45 ppb	270 ± 9.8 gh	311.3 ± 22.8 a	525.0 ± 62.9 cde	831.5 ± 21.7 hi
40HO * 45 ppb	317.8 ± 30.6 hi	834.0 ± 68.7 e	1108.3 ± 134.8 g	976.6 ± 63.9 ij
96 KG * 45 ppb	381.7 ± 0.4 j	246.3 ± 16.5 a	841.0 ± 75.1 f	760.5 ± 36.1 gh
201 KA * 45 ppb	161.7 ± 0.4 cde	427.0 ± 27.1 b	547.0 ± 62.4 de	1073.2 ± 54.2 j
Grand mean	220.61 ± 14.7	434.07 ± 21.5	610.56 ± 36.4	506.10 ± 39.3

Values represent the average of three replicates ± standard error of the mean (SEM). In a column, means followed by the same letters are not significantly ($p > 0.05$) different at $p \leq 0.05$ based on the LSD test; **MAT**, Months after transplanting; **ppb**, plants per bed; bed area = 1.8 m^2.

Table 2
Effect of accession and plant density on aboveground dry biomass (g) of *Cryptolepis sanguinolenta*.

Accessions	9 MAT	12 MAT	15 MAT	18 MAT
77 KAA	907.9 ± 79.0 b	1400.9 ± 158.8 ab	1118.7 ± 120.6 a	858.1 ± 86.9 a
176 KAA	624.3 ± 205.7 a	1660.1 ± 91.07 c	1347.2 ± 151.9 a	1045.9 ± 219.7 ab
15 DNN	1245.6 ± 92.0 c	1738.4 ± 144.7 c	1382.6 ± 131.2 a	1073.7 ± 139.6 b
40 HO	1247.9 ± 195.4 c	2382.2 ± 513.4 d	1267.8 ± 168.5 a	1198.2 ± 186.1 bc
96 KG	1247.7 ± 211.2 c	1522.0 ± 100.1 b	1245.9 ± 105.0 a	1282.6 ± 82.9 c
201 KA	1311.3 ± 70.9 c	1310.3 ± 84.0 a	1325.2 ± 93.4 a	1348.2 ± 210.8 c
Plant density (plants/bed)				
15 ppb	709.2 ± 77.0 a	1866.9 ± 286.9 b	1004.2 ± 69.1 a	682.5 ± 71.9 a
30 ppb	1110.6 ± 116.9 b	1553.9 ± 93.9 a	1461.8 ± 79.8 b	1281.1 ± 98.7 b
45 ppb	1472.6 ± 91.1 c	1586.2 ± 76.5 a	1377.7 ± 86.1 b	1439.8 ± 94.7 c
Plant density * Accessions				
77 KAA * 15 ppb	613.0 ± 92.4 b	780.0 ± 48.0 ab	1083.7 ± 201.2 cdef	729.5 ± 29.6 bc
176 KAA * 15 ppb	188.3 ± 27.6 a	1897.3 ± 66.3 h	806.7 ± 93.3 abc	317.8 ± 29.3 a
15 DNN * 15 ppb	924.3 ± 20.5 cd	2117.3 ± 87.6 i	955.7 ± 115.3 abcd	595.9 ± 103.3 ab
40 HO * 15 ppb	531.7 ± 39.5 b	4264.7 ± 174.9 j	753.0 ± 75.1 a	550.1 ± 133.4 ab
96 KG * 15 ppb	841.3 ± 54.0 c	1158.7 ± 121.5 cd	1309.3 ± 213.1 efgh	1163.5 ± 161.4 de
201 KA * 15 ppb	1156.3 ± 20.5 ef	983.3 ± 13.4 bc	111.8 ± 147.8 cdefg	738.1 ± 98.7 bc
77 KAA * 30 ppb	1041.3 ± 20.5 de	1770.3 ± 57.4 gh	1493.0 ± 57.7 hij	647.3 ± 29.4 ab
176 KAA * 30 ppb	239.3 ± 20.5 a	1753.0 ± 69.6 gh	1769.0 ± 57.7 ijk	1833.3 ± 29.4 f
15 DNN * 30 ppb	1517.0 ± 69.3 h	1915.7 ± 32.8 hi	1805.5 ± 89.2 jk	1219.1 ± 139.1 de
40 HO * 30 ppb	1459.0 ± 41.0 gh	757.0 ± 12.5 a	1201.5 ± 34.9 defgh	1238.7 ± 30.2 de
96 KG * 30 ppb	815.3 ± 65.6 c	1657.3 ± 44.0 fg	1024.0 ± 178.4 abcde	1468.4 ± 136.0 e
201 KA * 30 ppb	1591.3 ± 20.5 hi	1470.0 ± 57.7 ef	1477.8 ± 201.6 hi	1279.6 ± 287.3 de
77 KAA * 45 ppb	1069.3 ± 20.5 de	1652.3 ± 66.2 fg	779.3 ± 49.6 ab	1197.5 ± 29.6 de
176 KAA * 45 ppb	1445.3 ± 20.5 gh	1330.0 ± 57.7 de	1466.0 ± 150.5 hi	986.4 ± 29.5 cd
15 DNN * 45 ppb	1295.5 ± 81.1 fg	1182.3 ± 16.2 cd	1386.7 ± 68.3 fgh	1406.3 ± 154.3 e
40 HO * 45 ppb	1753.0 ± 220.5 i	2125.0 ± 77.8 i	1848.8 ± 174.3 k	1805.8 ± 36.5 f
96 KG * 45 ppb	2086.3 ± 20.5 j	1750 ± 49.3 gh	1404.5 ± 126.2 gh	1216.0 ± 106.8 de
201 KA * 45 ppb	1186.3 ± 20.5 ef	1477.7 ± 30.4 ef	1381.0 ± 85.4 fgh	2027.0 ± 147.8 f
Grand mean	1097.45 ± 69.4	1669.00 ± 103.6	1281.24 ± 52.3	1134.45 ± 67.5

Values represent the average of three replicates ± standard error of the mean (SEM). In a column, means followed by the same letters are not significantly ($p > 0.05$) different at $p \leq 0.05$ based on the LSD test; **MAT**, Months after transplanting; **ppb**, plants per bed; bed area, 1.8 m^2.

density of 30 ppb (Table 3).

3.2. Effect of accession and plant density on cryptolepine content

The results revealed that the growing period (months) did not significantly affect the cryptolepine content (mg) of 100 mg of dry root samples irrespective of the planting density or accession (Table 4).

However, the interaction between the two factors, accession and plant density significantly influenced the cryptolepine content (Fig. 1). The maximum cryptolepine content (2.08 mg/100 mg) was achieved in 96 KG+ 45 ppb, whereas the least (1.28 mg/100 mg) was obtained in 176KAA+30 ppb.

The observed significant effects of higher plant density on the content of bioactive agents such as cryptolepine in *C. sanguinolenta* are consistent with previous reports of similar effects in *Artemisia dracunculus* [39], *Ginkgo biloba* [11], *Astragalus membranaceus* [40] and *Satureja mutica* [35].

3.3. Effect of accession, plant density and growth periods on cryptolepine yield of C. sanguinolenta

Further analysis from the study revealed that cryptolepine yields (mg/bed) were significantly enhanced by the interaction between accession and plant density at each growth period (Table 5). At 45 ppb, where cryptolepine yield was highest, the average yield at the end of the study (18 months) period was 14.61 mg and ≈3.4-fold higher than 9-month grown plants (4.31 mg) (Table 5). This result highlighted the potential economic gain for producing this all-important plant under high plant density conditions to ensure maximum cryptolepine yield. Interestingly, there was a parallel relationship between cryptolepine yield and root biomass across all growing periods such that an increase in dry root biomass resulted in an increase in cryptolepine yield (Table 4). Hence, plant growth processes and substances that enhance root development and biomass accumulation are predicted to also enhance increased cryptolepine yield. This study revealed that increasing the plant density of *C. sanguinolenta* per unit area increases its economic yield which combines both cryptolepine synthesis and root biomass accumulation. The increase in cryptolepine yield in *C. sanguinolenta* plants grown under high plant density could be a result of the increased root biomass production. These findings are consistent with reports on Sahandi, Arabian coffee, Bakhtiari and Safflower where significant improvements in plant biomass production and essential oil yield were found [35, 41–44].

Table 3
Effect of accession and spacing on the dry shoot-to-root ratio of *Cryptolepis sanguinolenta.*

Accessions	9 MAT	12 MAT	15 MAT	18 MAT
77 KAA	5.15 ± 0.56 ab	3.45 ± 0.26 a	2.22 ± 0.14 a	3.45 ± 0.27 b
176 KAA	5.99 ± 0.23 c	3.84 ± 0.35 a	2.32 ± 0.21 a	7.65 ± 4.17 c
15 DNN	4.69 ± 0.36 a	3.56 ± 0.17 a	2.86 ± 0.27 a	2.25 ± 0.26 a
40 HO	5.63 ± 0.40 bc	4.91 ± 1.08 b	1.82 ± 0.15 a	2.01 ± 0.11 a
96 KG	4.61 ± 0.26 a	4.90 ± 0.63 b	2.50 ± 0.66 a	1.95 ± 0.13 a
201 KA	5.74 ± 0.44 bc	3.64 ± 0.39 a	2.38 ± 0.21 a	2.03 ± 0.11 a
Plant density (plants/bed)				
15 ppb	5.30 ± 0.28 a	4.76 ± 0.54 c	2.84 ± 0.33 b	2.22 ± 0.12 a
30 ppb	5.16 ± 0.27 a	3.42 ± 0.18 a	1.96 ± 0.12 a	5.34 ± 2.23 b
45 ppb	5.45 ± 0.32 a	3.97 ± 0.37 b	2.25 ± 0.14 a	2.11 ± 0.13 a
Plant density * Accessions				
77 KAA * 15 ppb	5.25 ± 0.42 cdef	2.51 ± 0.25 a	1.79 ± 0.07 abcd	2.46 ± 0.18 bcdef
176 KAA * 15 ppb	6.48 ± 0.24 fghi	4.24 ± 0.08 cd	2.52 ± 0.45 bcde	2.67 ± 0.69 cdef
15 DNN * 15 ppb	3.62 ± 0.61 ab	3.82 ± 0.06 bcd	3.70 ± 0.16 ef	1.98 ± 0.10 abcd
40 HO * 15 ppb	6.80 ± 0.45 ghi	9.05 ± 1.01 f	1.53 ± 0.08 ab	2.10 ± 0.32 abcde
96 KG * 15 ppb	4.36 ± 0.36 abcd	4.67 ± 0.30 d	4.55 ± 1.40 f	1.85 ± 0.00 ab
201 KA * 15 ppb	5.29 ± 0.10 cdef	4.26 ± 1.21 cd	2.95 ± 0.23 de	2.27 ± 0.13 abcdef
77 KAA * 30 ppb	7.01 ± 0.16 hi	3.95 ± 0.13 bcd	2.57 ± 0.00 bcde	5.12 ± 0.21 g
176 KAA * 30 ppb	5.28 ± 0.54 cdef	4.38 ± 0.84 d	1.90 ± 0.00 abcd	17.43 ± 2.46 h
15 DNN * 30 ppb	5.66 ± 0.36 efg	3.00 ± 0.12 ab	2.13 ± 0.24 abcd	3.05 ± 0.51 f
40 HO * 30 ppb	4.59 ± 0.56 bcde	3.01 ± 0.04 abc	2.25 ± 0.31 abcd	2.07 ± 0.01 abcde
96 KG * 30 ppb	4.01 ± 0.36 abc	2.90 ± 0.07 ab	1.28 ± 0.19 a	2.41 ± 0.16 bcdef
201 KA * 30 ppb	4.43 ± 0.06 abcde	3.17 ± 0.12 abc	1.64 ± 0.02 abc	1.95 ± 0.26 abcd
77 KAA * 45 ppb	3.21 ± 0.10 a	3.89 ± 0.22 bcd	2.29 ± 0.25 abcd	2.73 ± 0.14 def
176 KAA * 45 ppb	6.21 ± 0.19 fgh	2.88 ± 0.20 ab	2.55 ± 0.45 bcde	2.87 ± 0.20 ef
15 DNN * 45 ppb	4.79 ± 0.13 bcde	3.84 ± 0.29 bcd	2.73 ± 0.42 cde	1.70 ± 0.23 ab
40 HO * 45 ppb	5.51 ± 0.42 def	2.57 ± 0.13 a	1.69 ± 0.15 abc	1.87 ± 0.16 abc
96 KG * 45 ppb	5.47 ± 0.06 def	7.14 ± 0.28 e	1.67 ± 0.00 abc	1.59 ± 0.07 a
201 KA * 45 ppb	7.50 ± 0.15 i	3.50 ± 0.28 abcd	2.56 ± 0.14 bcde	1.88 ± 0.04 abc
Grand mean	5.23 ± 0.16	4.05 ± 0.24	2.35 ± 0.13	3.64 ± 0.79

Values represent the average of three replicates ± standard error of the mean (SEM). In a column, means followed by the same letters are not significantly ($p > 0.05$) different at $p \leq 0.05$ based on the LSD test; **MAT**, Months After Transplanting; **ppb**, plants per bed; bed area, 1.8 m^2.

Table 4
Mean dry root weight, dry shoot weight, dry shoot-to-root ratio, cryptolepine concentration and cryptolepine yield at different harvest periods.

Months	Dry Root Weight (g/bed)	Dry Shoot Weight (g/bed)	Dry Shoot-to-Root Ratio	Cryptolepine Conc. (mg/100 mg) of Dry root	Cryptolepine Yield (g/bed)
9	220.4 ± 14.7 a	1097.5 ± 69.4 b	5.24 ± 0.17 c	1.46 ± 0.06 a	3.36 ± 0.29 d
12	434.1 ± 21.5 b	1669.0 ± 104.0 a	4.05 ± 0.24 b	1.82 ± 0.08 a	7.75 ± 0.43 c
15	610.6 ± 36.2 c	1281.2 ± 52.1 b	2.35 ± 0.13 a	1.75 ± 0.05 a	10.33 ± 0.53 a
18	496.6 ± 39.3 b	1134.5 ± 67.5 b	3.64 ± 0.79 b	1.70 ± 0.06 a	8.95 ± 0.81 b
Mean	440.4 ± 17.7	1295.6 ± 40.57	3.82 ± 0.22	1.68 ± 0.03	7.60 ± 0.34

Values represent the average of three replicates ± standard error of the mean (SEM). In a column, means followed by the same letters are not significantly ($p > 0.05$) different at $p \leq 0.05$ based on the LSD test.

3.4. Effect of accession, plant density and growth periods on benefit-cost ratio of C. sanguinolenta

From the benefit-cost analysis performed, cultivating *C. sanguinolenta* at 45 plants/bed (25,920 plants/acre) for a cropping period of 18 months gave the highest total revenue of GHC 27,639.55 (Fig. 4). This was followed by 30 plants/bed (17,280 plants/acre) which gave a total revenue of GHC 26,016.36 in 15 months (Fig. 3) while 15 plants/bed (8640 plants/ha) gave the lowest total revenue of GHC 6835.20 in 9-months (Fig. 2). The net profit generated from cultivating *C. sanguinolenta* was highest at 45 plants/bed (25,920 plants/acre) giving GHC 19,391.55 in 18 months (Fig. 4) followed by a GHC 17,768.36 net profit generated at 30 plants/bed (17,280 plants/acre) in 15-months cropping period (Fig. 3). The lowest net profit was generated from cultivating *C. sanguinolenta* at 15 plants/bed (8640 plants/ha) (Fig. 2). The economic analysis revealed that growing of *C. sanguinolenta* at 45 plants/bed (25,920 plants/acre) for 18 months recorded the highest benefit-cost ratio of 3.4 (Tables 2 and 3) while the least benefit-cost ratio was recorded from 15 plants/bed (8640 plants/ha) (0.8) in the 9-months cropping period. Across the cropping periods, the total revenue increased as the planting density increased.

Similar trends were reported in *Ipomoea batatas* by Idoko et al. [45] and Uzoigwe et al. [34], where it was observed that the higher the planting density the higher the net return per hectare.

To determine the potential benefits of farmers' intensive cultivation of *C. sanguinolenta* as a cash crop and its connection with production costs, a benefit-cost ratio analysis was performed. The 3.4 benefit-cost ratio obtained or '45 plants/bed' after 18 months of

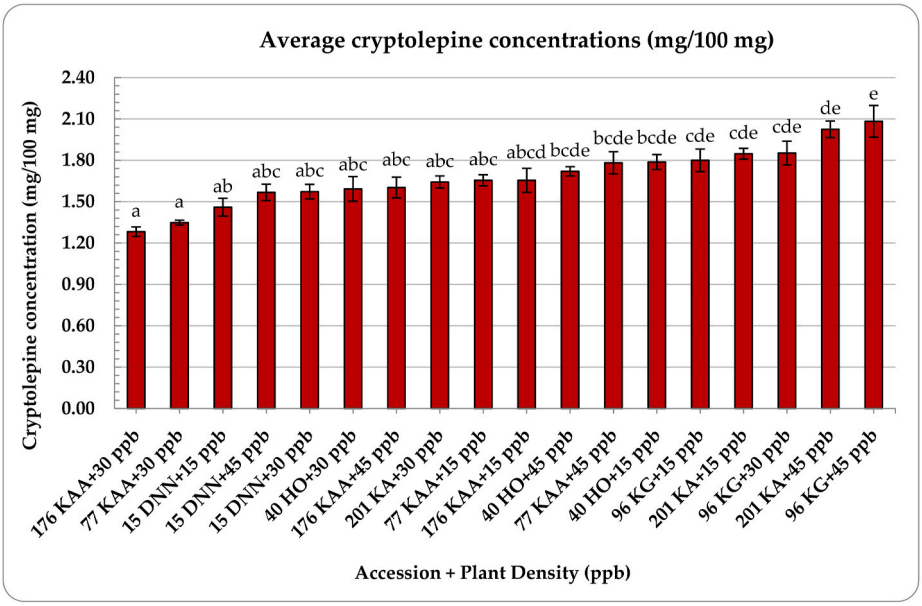

Fig. 1. Cryptolepine concentration in roots of *Cryptolepis sanguinolenta* in response to the interaction between accession and plant density. The 15 ppb, 30 ppb and 45 ppb denote the planting densities/plants per bed at which each accession was planted. Bars represent the mean of three replicates, and error bars are the SEM. Bars followed by different letters are significantly different at $p \leq 0.05$ based on the LSD test; **ppb**, plants per bed; bed area, 1.8 m^2.

Table 5
Effect of accession and plant density on average cryptolepine yield (mg/bed) of *Cryptolepis sanguinolenta*.

Accessions	9 MAT	12 MAT	15 MAT	18 MAT
77 KAA	3.23 ± 0.61 bc	6.30 ± 0.34 a	7.61 ± 0.69 a	5.21 ± 1.40 a
176 KAA	0.84 ± 0.23 a	7.40 ± 0.60 bc	10.36 ± 1.06 c	4.09 ± 1.04 a
15 DNN	3.16 ± 0.13 b	7.98 ± 1.13 c	9.31 ± 1.67 bc	9.53 ± 2.03 b
40 HO	5.05 ± 1.05 e	7.27 ± 1.39 b	13.25 ± 1.76 d	10.20 ± 2.14 b
96 KG	3.83 ± 0.53 cd	9.96 ± 1.46 d	12.23 ± 1.41 d	9.79 ± 1.11 b
201 KA	4.03 ± 0.42 d	7.58 ± 1.00 bc	9.24 ± 0.83 b	14.91 ± 2.46 c
Plant density (plants/bed)				
15 ppb	2.11 ± 0.26 a	7.24 ± 0.58 a	7.78 ± 0.75 a	5.32 ± 0.60 a
30 ppb	3.65 ± 0.59 b	7.52 ± 1.00 a	12.20 ± 0.72 c	6.93 ± 1.13 b
45 ppb	4.31 ± 0.44 c	8.49 ± 0.66 b	11.02 ± 1.15 b	14.61 ± 1.46 c
Plant density * Accessions				
77 KAA * 15 ppb	2.34 ± 0.17 cd	5.43 ± 0.08 cd	8.77 ± 1.31 cde	4.13 ± 0.40 bc
176 KAA * 15 ppb	0.32 ± 0.01 a	8.09 ± 0.49 ef	7.89 ± 2.49 cd	4.32 ± 2.04 bc
15 DNN * 15 ppb	2.67 ± 0.11 cde	11.54 ± 0.14 gh	3.83 ± 0.13 a	3.79 ± 0.60 bc
40 HO * 15 ppb	1.21 ± 0.16 ab	7.49 ± 0.39 e	11.66 ± 1.91 fg	4.99 ± 1.79 cd
96 KG * 15 ppb	2.83 ± 0.31 cde	6.12 ± 0.47 d	6.97 ± 0.76 bc	7.79 ± 1.65 ef
201 KA * 15 ppb	3.30 ± 0.25 de	4.75 ± 1.21 bc	7.57 ± 0.67 cd	6.9d ± 1.00 e
77 KAA * 30 ppb	1.75 ± 0.04 bc	5.98 ± 0.20 d	8.65 ± 0.15 cd	2.21 ± 0.08 ab
176 KAA * 30 ppb	0.45 ± 0.01 a	5.32 ± 0.73 cd	12.64 ± 0.47 gh	1.06 ± 0.03 a
15 DNN * 30 ppb	3.50 ± 0.06 e	8.53 ± 0.49 ef	14.77 ± 1.75 ij	8.09 ± 1.48 ef
40 HO * 30 ppb	7.74 ± 0.52 g	2.39 ± 0.07 a	9.10 ± 0.79 de	7.84 ± 2.03 ef
96 KG * 30 ppb	2.85 ± 0.06 de	15.45 ± 0.96 i	16.00 ± 0.27 j	7.91 ± 0.35 ef
201 KA * 30 ppb	5.59 ± 0.42 f	7.46 ± 0.37 e	12.03 ± 0.89 fgh	14.50 ± 0.73 gh
77 KAA * 45 ppb	5.61 ± 0.24 f	7.51 ± 0.42 e	5.40 ± 0.55 ab	9.30 ± 2.99 f
176 KAA * 45 ppb	1.75 ± 0.01 bc	8.78 ± 0.49 f	10.55 ± 1.20 ef	6.90 ± 0.61 de
15 DNN * 45 ppb	3.30 ± 0.07 de	3.86 ± 0.26 b	9.33 ± 0.58 de	16.69 ± 1.93 hi
40 HO * 45 ppb	6.19 ± 1.16 f	11.93 ± 0.51 h	18.99 ± 2.52 k	17.76 ± 1.60 i
96 KG * 45 ppb	5.80 ± 0.58 f	8.32 ± 0.71 ef	13.72 ± 1.05 hi	13.67 ± 0.84 g
201 KA * 45 ppb	3.21 ± 0.24 de	10.55 ± 1.36 g	8.10 ± 1.12 cd	23.31 ± 1.83 j
Grand mean	4.31 ± 0.60	8.50 ± 0.70	11.02 ± 1.79	14.61 ± 1.58

Values represent the average of three replicates ± standard error of the mean (SEM). In a column, means followed by the same letters are not significantly ($p > 0.05$) different at $p \leq 0.05$ based on the LSD test; **MAT**, Months after transplanting; **ppb**, plants per bed; bed area, 1.8 m^2.

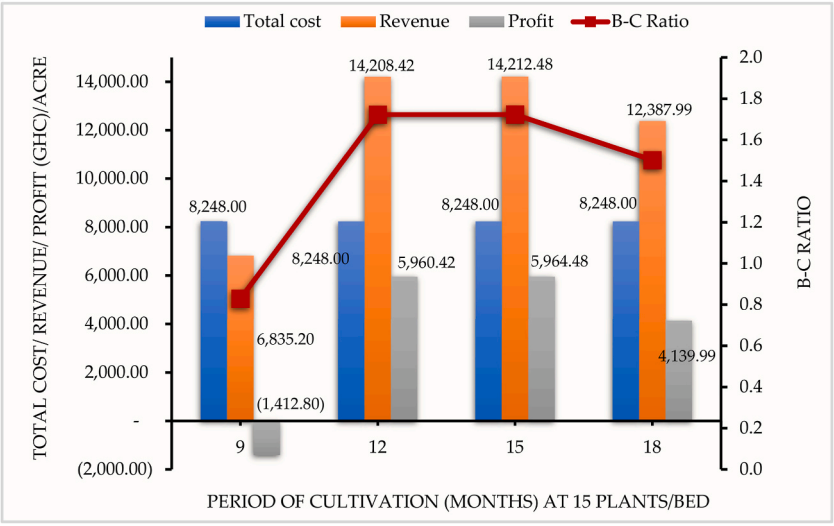

Fig. 2. Benefit-cost ratio analysis of the *C. sanguinolenta* at 15 ppb for a 9–18 month cropping period.

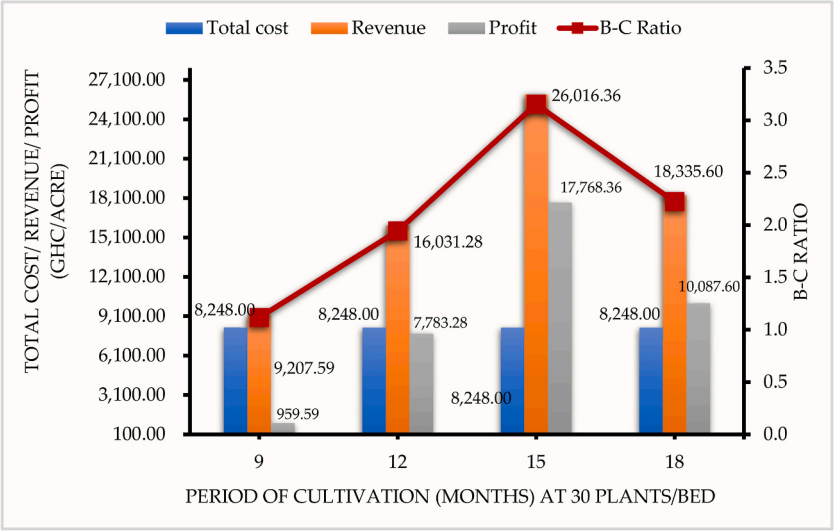

Fig. 3. Benefit-cost ratio analysis of the *C. sanguinolenta* at 30 ppb for a 9–18 month cropping period.

cultivation implied the benefit of farmer-intensive cultivation outweighed the cost, such that for every GHC1.00 invested in the cultivation of *C. sanguinolenta* at 45 plants/bed (25,920 plants/acre) planting density, GHC3.4 is realizable in benefits. Similar findings were reported in *Manihot esculenta* [46] and *Ipomoea batatas* [47] where a benefit-cost ratio greater than 1 was recorded.

4. Conclusions

The study showed that accession and plant density at harvest positively affected the general development and cryptolepine yield in *C. sanguinolenta*. Cultivation at high plant densities increased root biomass yield which has been determined as the most economic part of the plant. The cryptolepine content and yield were maximized under high plant densities and the 9-month growth period was confirmed as optimal for harvesting the plant by plant-medicine practitioners since the active ingredient concentration is not affected by the growth period. This finding is crucial for the plant industry since it further clarifies the growth and maturation stage of the roots at which there is maximum active ingredient bioactivity. However, the dry root weight and cryptolepine yield were highest at 18 months. This was confirmed as optimal for harvesting the roots by commercial farmers since revenue generation from the cultivation of *C. sanguinolenta* heavily depends on sales of dry roots and cryptolepine yield. From this study, the benefit-cost ratio calculation revealed that farmer-intensive cultivation of *C. sanguinolenta* was profitable and the highest benefit-cost ratio was obtained from 45 plants/bed (25,920 plants/acre) cultivated for 18 months. Similarly, the highest returns were recorded from selling dried roots

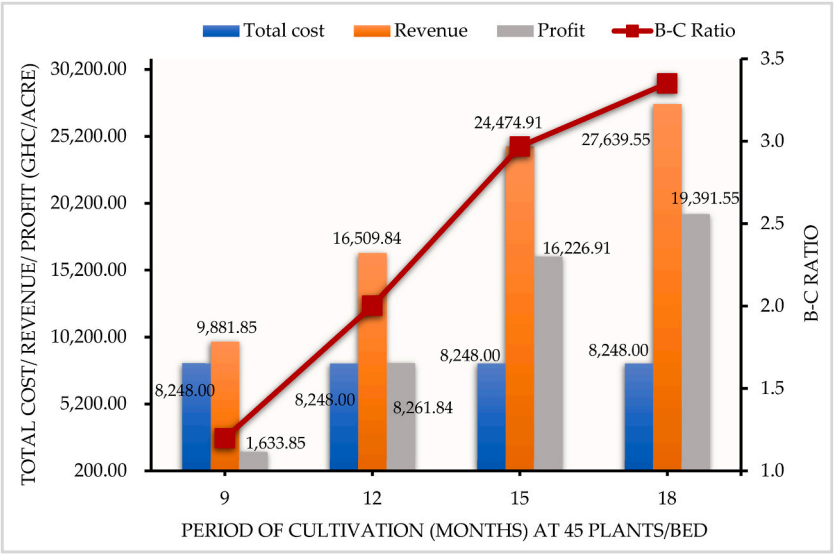

Fig. 4. Benefit-cost ratio analysis of the *C. sanguinolenta* at 45 ppb for a 9–18 month cropping period.

produced at the same plant density and cultivation period. Therefore, cultivation of *C. sanguinolenta* at 45 plants/bed (25,920 plants/acre) for 15–18 months is the most sustainable approach to ensure its efficient commercial production and will be of great interest to farmers as the most profitable venture in improving their rural livelihoods.

Our research contributes to a better understanding of *C. sanguinolenta's* cultivation. The exclusive focus on the concentration of the active ingredient cryptolepine, potentially neglecting the presence and influence of other essential indole alkaloids in *C. sanguinolenta*, is because cryptolepine is the major alkaloid found in the roots of *C. sanguinolenta* and has been the reference for all previous agronomic studies [15,31]. The study failed to capture the synergistic effects of the other indole alkaloids because although present their contribution to the therapeutic outcomes of the plant species is still unknown limiting the comprehensive understanding of the plant's medicinal properties. Further studies are required to delve into the specific roles and effects of the complex interplay of the different alkaloids in *Cryptolepis sanguinolenta* for a more comprehensive understanding.

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Jacqueline Naalamle Amissah: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Frank Opoku-Agyemang:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Freda Elikplim Asem:** Writing – review & editing, Methodology, Data curation. **Dorcas Osei-Safo:** Writing – review & editing, Supervision, Methodology. **Ivan Addae-Mensah:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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