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Research Article

Effect of varying levels of achichi (*Cannabis sativum* L.) seed oil extract in the inhibition of bacteria spot disease of scotch pepper (*Capsicum annum* L.)

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Abstract

Negative food safety reports over the use of synthetic pesticides in controlling bacteria disease of pepper caused by *Xanthomonas campestris pv* is increasing in Nigeria, hence the need for friendly options. An experiment was carried out at the Teaching and Research Farm of Rufus Giwa Polytechnic, Owo, located on latitude 7^0 12N and longitude 5^0 35E at 350m above sea levels, to evaluate the effect of varying levels of achichi (Cannabis sativum L.) seed oil extract in the inhibition of bacteria spot disease of scotch pepper (Capsicum annum L.). Treatments include; Positive control (Synthetic Rindomil gold), Negative control (distill water), 2 ml ASO (Achichi seed oil), and 4ml ASO (Achichi seed oil) which were randomly distributed into plots, arranged in a Randomized Complete Block Design (RCBD). Results showed that the application of ASO had bactericidal inhibition potential against the bacteria spot diseases comparable to the synthetic ridomil powder. Application of between 2 – 4 ml / 1 liter of water of ASO was found to significantly influence the general performance of sprayed pepper in the study area. Further investigation should be carried out to determine the right volume between the ranges of 2 ml – 4 ml of ASO that can effectively control the bacteria spot disease in the study area.

Introduction

Pepper cultivation in Nigeria has intensified in recent years with a total production of about 410,033 tons of pepper fruits for both processing and fresh market [1] and for commercial and home consumption [2,3]. It constitutes 7% of the total horticultural produce and 14% of the total vegetables grown in Nigeria [1]. The fruit is always in high demand since it is consumed by nearly all households. It is rich in capsaicin which is [3], and also minerals and vitamins A, C, and E [4].

The largest percentage of peppers produced in Nigeria is under open fields where they are vulnerable to diseases and pests [5]. Diseases such as bacteria leaf spot caused by *Xanthomonas campestris pv. vesicatoria* affect the quality, quantity, and profitability of pepper [1], more importantly when environmental conditions are conducive [6] and losses can be close to 100% [7]. The symptoms appear as spot lesions on leaves, stems, and fruit, and occur at all stages of plant growth [8].

Farmers rely on synthetic fungicides in the control of disease on the field but, there are reported cases of toxicity and residue retention in the food products [9] and to beneficial and non-target pollinators [10] as a result of synthetic chemical misuse.

The worldwide trend to explore organic/biological pesticides as alternative options to synthetic ones is gaining popularity [11] mainly because of its biodegradable, nonpoisonous, and safe to non-target pollinators and without harmful residues in food products [12]. Several plant compounds have been reported to control bacterial and fungal pathogens in vitro and in vivo domains [13]. The plant compounds contain essential oils (aromatic and volatile liquids) which have abundant bioactive compounds with antibacterial activity [7,14,15] that

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can replace toxic synthetic bactericides. The current study is therefore aimed at investigating the antimicrobial activity of varying levels of Achichi (*Cannabis sativa* L.) seed oil (ASO) extract against pepper bacterial spot diseases under field conditions.

Materials and methods

Location of the study

The study was carried out at the Teaching and Research Horticultural Production Garden, Rufus Giwa Polytechnic, Owo, Ondo state, Latitude 7^{o} 12^{N} and Longitude 5^{o} 35^{E}, at 300m above sea level. Owo falls under the Guinea Savannah transition zone with a bimodal rainfall pattern that varies from 1200 mm to 2000 mm and spreads from April to July, attaining the first peak in July and September to November with a noticeable peak in September. The maximum and minimum temperatures of the area average are 29 ˚C and 15 ˚C and is also even throughout the year. The relative humidity is 60% during the dry season but increases to 80% in the rainy season

Collection of diseased pepper plant materials

Sampling was done in Horticultural Production Garden, Rufus Giwa Polytechnic, Owo. Pepper leaves with bacteria spot symptoms was randomly collected inside cool boxes by a physical examination in the laboratory and refrigerated at 4 ˚C for processing and further analysis.

Isolation of the pathogens

V8 and Potato Dextrose Agar (PDA) [16] were the standard media used for isolation of *Xanthomonas campestris pv. vesicatoria* from the diseased pepper samples. The pepper leaves bearing bacteria spot symptoms were first washed under clean running tap water and then surface sterilized in 1% sodium hypochlorite for three minutes. They were rinsed in three changes of sterilized distilled water and blotted dry using sterilized blotting paper. A sterilized scalpel was used to cut infected leaf tissues of 3 × 3 mm size toward the healthy tissues where the pathogens were suspected to be more active.

The surface sterilized tissues were directly plated on the sterilized PDA and V8 agar for bacteria spots and then incubated in the laboratory at room temperature (27 ˚C) for three days. The colonies were then subjected to single spore isolation and subcultured on the media to obtain pure strains for identification. The isolates were identified using morphological microscopic and macroscopic features and compared with established identification keys like gram stain, oxygen requirement, spore formation, and shape. The isolates were then maintained on plates awaiting their inoculation on pepper plants in the field.

Extraction of Achichi Seed Oils (ASO)

Clean seeds were poured inside a blender (industrial types XL Kenwood Chef Product) and were blended into a powder. The powder was then poured inside a rubber container filled with hexane and allowed to stay for 36 hours, to get the desired liquid. The achichi seed powder and hexane were poured into a muslin cloth and squeezed with hand to get the extraction of achihi oil needed and the remaining residue left inside the muslin cloth was disposed of. The liquid was allowed to settle for 22 hours and then separated. A retort stand was used to hold the soxhlet, a condenser was connected to the soxhlet to cover it, a boiling flask was connected to the soxhlet to cover it, mantle machine was plugged in for heating. The achichi oil was poured inside the boiling flask and put on top of the mantle machine for boiling. It was made to boil for 25 minutes while connected to run through condenser with running water for cooling the hexane pressure. The soxhlet annexed the drops of hexane from the condenser and the extracted achichi oil remains in the boiling flask. The oil was later poured inside a beaker and put inside the oven for 45 minutes to drain out remnants of hexane inside the extract. The oil was later bottled and stored in the refrigerator till application in the experiment.

Experimental design and layout

The experiment was laid out in a Randomized Complete Block Design (RCBD) with four treatments replicated four times. There were six main plots in a block representing the plants inoculated by the pathogens (*Xanthomonas campestris pv*. vesicatoria). The three treatments comprised 'the control, achichi seed oils at 2 and 4 ml concentration, the positive control (Ridomil Gold synthetic fungicide), and the negative control (distilled water). Ridomil Gold is a curative fungicide manufactured by Syngenta (https://www.syngenta.co.ke/product/crop-protection/ridomil-gold-mz-68-wg) and comprises Metalaxyl-M 40 g/Kg and Mancozeb 640 g/Kg formulated as wettable granules. The test pepper variety was Scotch bonnet pepper (Rodo) bought from the local market in Owo. It was not specified as being resistant to bacteria spot of pepper.

Before planting, the land was tilled, using a hand hoe, and well-decomposed manure was worked into the soil. The land was irrigated to improve the moisture status of the soil. One-month-old pepper seedlings were transplanted in the field.

Preparation of inoculum

Fourteen-day-old cultures of Bacteria spot disease were used as a source of the spores. The spore suspension was prepared by adding 5 ml of sterile distilled water to a pure 14-day-old culture in a Petri dish. Dislodging of spores was done with a glass rod and the content was sieved using a three-layer cheesecloth to remove the mycelia. The hemocytometer slide was used under a microscope to ascertain the spore suspension concentration and then standardized to 1 × 106 spores per millimeter with sterile water. The inoculum was stored in the refrigerator, at 4 ˚C, awaiting inoculation.

Inoculation and application of treatments

Inoculation was done on actively growing pepper plants two weeks after transplanting by spraying every plant with 20 ml of the inoculum using a hand sprayer. Symptoms of disease development began to appear on the 7th day after inoculation. In the preparation for spraying, 2 and 4 ml of achichi seed oil were first mixed separately with sterile distilled water to up

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to 500 ml thoroughly. Ridomil Gold solution was prepared following the manufacturer's instructions of 2.5 g per liter of water.

Subsequent applications were done after every ten days up to the harvesting stage of pepper fruits.

Data collection and analysis

Disease severity was scored per treatment every ten days using a 0–5 scale [17] based on the size and number of lesions on the infected leaves as follows:

i. 0 = healthy (no visible lesions on the leaf)

ii. 1 = up to 10% of the infected leaf area

iii. 2 =11%–25% of the infected leaf area

iv. 3 = 26%–50% of the infected leaf area

v. 4 = 51%–75% of the infected leaf area

vi. 5 = more than 75% of the infected leaf area

The disease scales were converted into percentages for each plant using the formula described by Chaerani and Voorrips [18] as provided in the equation.

$$\text{Disease severity} = \frac{\text{Sum of all ratings} \times 100}{\text{No. of leaves sampled} \times \text{maximum disease scale}}$$

The disease severity of each plant was determined according to the average of all leaves of a plant counted based on visual assessment [19].

Data was also collected on growth and yield parameters including plant height, number of branches/plant, number of leaves/plants, days to 50% flowering, and number and weight of marketable fruits.

All the data was subjected to Analysis of Variance (ANOVA) using SPSS version 2019 and separation of means was done, using LSD (Least Significant Difference) at a 95% level of confidence.

Results and discussions

Severity of bacteria spot disease on inoculated pepper plant

The severity of bacteria spot disease on pepper plants inoculated with *Xanthomonas campestris* kept increasing throughout the experiment (Table 1). At 2 WAT, the severity of the disease reduced significantly ($p > 0.05$) with the application of achichi seed oil. The achichi seed oils at 4 ml concentration gave similar results to the synthetic fungicide (Ridomil Gold®) used as a positive control. The two concentrations (2 ml and 4 ml) varied significantly with the negative control (distilled water). For both concentrations, disease severity was significantly reduced. At low concentrations of achichi seed oil (2 ml.), resumption of inhibition commenced late, while the severity continued to increase in the negative control treatment.

Effects of achichi seed oils on the number of leaves, plant height, and number of branches of pepper inoculated with bacteria spot disease at 2,4,6,8 and 10 WAT

Results showed that there was no significant ($p > 0.05$) difference across all treatments in the number of leaves at 2 and 4 WAT. At 6, 8, and 10 WAT, significant differences ($p < 0.05$) were observed under different treatment levels. Control treatment recorded the least number of leaves while positive control and two levels of achichi seed oil treatments recorded significantly high values. There was no significant difference in number of leaves at positive control and the two levels of ASO at 6, 8, and 10 WAT. Achichi seed oil at 4 ml (19.58) > 2 ml (18.15) > Ridomil gold (18.11) and > control (12.58) (Table 2).

The pepper plants inoculated with bacteria spot disease did not show significant variation in height across all treatments at 2 WAT. At 4 WAT the pepper plants under control treatment showed significantly ($p < 0.05$) lower plant height (3.41 cm) than all the other treatments. Positive control, 4 ml and 2 ml of achichi oil treatments did not show significant variation in plant height. At 6 WAT the plant height varied significantly ($p < 0.05$) among the treatments with plants in the control treatment being shortest in the plants treated with 4 ml, 2 ml, and Ridomil gold (control). Pepper plants sprayed with 2 ml achichi seed oil and positive control (Rindomil g) did not vary significantly in plant height, but 4 ml was significantly higher from both the positive control and 2 ml treatments (Table 3).

There was a significant influence of treatments on bacteria spot disease on the number of branches of pepper plants across all stages of measurement ($p < 0.05$). Among treatments, the negative control recorded the lowest number of branches while pepper plants sprayed with 4 ml of achichi seed oil showed an increased number of branches. There was no significant

Table 1: Effect of achichi seed oil on disease severity of inoculated pepper plant treatments.

Age	Control	Rindomin G	ASO 2ml	ASO 4ml	LSD
2 WAT	19.87*	10.06ns	10.02ns	10.11ns	0.45
4 WAT	40.52**	9.27*ns	9.45*	9.02ns	0.28
6 WAT	57.27*	7.75ns	7.82ns	7.38ns	1.18
8 WAT	62.69*	7.23ns	7.55ns	7.22ns	1.02
10 WAT	70.06*	7.07ns	7.26ns	7.10ns	0.98

NOTE: **: Very Significant; *: Significant; ASO: Achichi Seed Oil; LSD: Least Significant Difference; WAT: Week After Transplanting.

Table 2: Effect of achichi seed oil on disease severity on the number of leaves of an inoculated pepper plant Treatments.

Age	Control	Rindomin G	ASO 2ml	ASO 4ml	LSD
2 WAT	4.16ns	4.16ns	4.17ns	4.15ns	0.52
4 WAT	7.23ns	7.55ns	7.45ns	7.56ns	0.42
6 WAT	10.65ns	16.02*	15.86*	16.03*	1.00
8 WAT	12.00ns	17.16*	17.12*	17.21*	0.63
10 WAT	12.58ns	18.11*	18.15*	19.58*	1.00

NOTE: **: Very Significant; *: Significant; ASO: Achichi Seed Oil; LSD: Least Significant Difference; WAT: Week After Transplanting.

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difference in number of branches on plants treated with 2 ml of achichi seed oil and positive control treatments (Table 4).

Effects of achichi seed oils on days to 50% flowering of pepper inoculated with bacteria spot disease

The treatments significantly ($p < 0.05$) influenced the number of days to 50% flowering on the pepper inoculated with bacteria spot disease. Treatment at 4 ml of achichi seed oil had significantly shorter days to attain a 50% flowering date followed by the positive control, then 2 ml. The negative control treatments took the longest time to attain a 50% flowering date concerning the other treatments (Table 4).

Effects of achichi seed oil on number of fruit and fruit weight per plant of pepper inoculated with bacteria spot disease

The number of pepper fruits produced varied significantly ($p < 0.05$) among the treatments (Table 4). Among pepper plants inoculated with bacteria spot disease, the negative control recorded the lowest number of fruits per plant. The number of fruits per plant increased with the application of positive control (Rindomin G = 5.88) and achichi seed oil extracts (4 ml = 5.99) and (2 ml = 5.86). There was no significant ($p > 0.05$) difference among treatments. Also, the application of achichi seed oil at 2, 4ml, and positive control (ridomil gold) on pepper plants significantly influenced the fruit weight of pepper than the negative control. Application of achichi seed oil at 4 ml increased fruit weight more significantly than positive control (Ridomil Gold) and 2 ml. Values of positive control and 2 ml were not significantly ($p > 0.05$) different levels (Table 4).

Discussion

Disease severity was not significant on the number of leaves at 2 – 4 WAT. The reason could be attributed to the inherent genetic resistance of the pepper plant against the disease during the formative stage of the pathogen and also that the environment may not compromise with the quick establishment of the pathogen at the time of inoculation [20]. At 6 – 10 WAT disease severity was high on pepper plants inoculated with bacteria spots under negative control, reducing the plant vegetative growth (number of leaves, plant height, and number of branches). Pepper plants inoculated with bacteria spot under positive control (Rindomil gold) and the two levels of achichi seed oil (2 and 4 ml) had low severity of disease infection, hence increasing vegetative growth (number of leaf, plant height, and number of branches), showing that the disease suppression mechanism, though varied with application level of the treatments, were effective. Kagale, et al. [21] pointed out that the mechanism of disease suppression may be either by active antimicrobial compounds acting on the pathogen directly by suppressing bacteria growth through inhibition of cell division, destroying their membranes via antioxidant activity (mitigating oxidizing potential) causing structural deviation and leakages, thus lowering disease development. This study showed that the antibacterial effects of achichi seed oil (ASO) reduced the chance of the pathogen developing resistant traces after application due to disruption of bacteria linking and coordination of virulent factors [15]. This was comparable to that of Ridomil Gold synthetic fungicide [1,22].

Flowering accounted for fruiting and yield of crops. This study showed that ASO efficacy was reflected in the attainment of Days to 50% flowering stage, fruit, and yield of pepper when compared with the untreated control. Late resumption of days to 50% flowering stage of pepper plant under negative control was an indication that the pathogen had implicated the physiological and biological processes of growth of the plant thereby affecting flowering formation. On the other hand, plants under positive control (Rindomil gold) and the two levels of ASO assumed 50% flowering stage early which led to the production of more flowers that eventually increased fruits and yield of pepper, which showed the capacity of ASO in controlling bacteria spot disease of pepper. This is probably due to the discharge of antioxidants [Tocotrienols] [23], antibacterial [carotenoids e.g. bixin and norbixin, [24]] anti-inflammatory and Flavonoids [25]. Essential oil, according to Din, et al. [26] contains secondary metabolites such as organosulfur, phenols, and alkaloids which are antimicrobial and reduce the chance of pathogens causing disease in plants. This corroborated the theory of Naing, et al. [9] that some organic pesticides induce disease resistance systems of the plant which lead to healthy growth of the plants and thus better production. Nashwa and Abo-Elyousr, [[22]; and Ahmad, et al. [27] also showed that the Eucalyptus camaldulensis (eucalyptus), Ocimum basilicum (sweet basil), Allium sativum (garlic), Nerium oleander (oleander), Datura stramonium (jimsonweed), and Azadirachta indica (neem) plant extracts boosted the yield of tomato as compared to the control. However, restraints to ASO production are confined in availability, accessibility, and regulatory system, which may hinder its use and adoption in the study area [25], as compared to essential oils from other plant extract.

Table 3: Effect of achichi seed oil on disease severity on plant height of inoculated pepper plant treatments.

Age	Control	Rindomin G	ASO 2ml	ASO 4ml	LSD
2 WAT	3.41ns	5.09*	4.98*	5.10*	0.66
4 WAT	5.00ns	8.62*	7.76*	8.64*	0.82
6 WAT	10.55ns	14.36*	13.84*	14.73*	1.02
8 WAT	13.18ns	15.00*	14.98ns*	15.25*	0.78
10 WAT	17.56ns	19.93*	20.02*	20.06*	1.28

NOTE: **: Very Significant; *: Significant; ASO: Achichi Seed Oil; LSD: Least Significant Difference; WAT: Week After Transplanting.

Table 4: Effect of achichi seed oil on disease severity on growth and yield of inoculated pepper plant at 50% flowering stage and harvest Treatments.

Parameters	Control	Rindomin G	ASO 2ml	ASO 4ml	LSD
No of branches	2.63ns	3.31*	3.28*	3.53*	0.50
DTO 50% Flowering	62.00ns	56.17*	56.05*	57.23*	2.15
No of fruit	3.68ns	5.88*	5.86*	5.99*	0.66
Weight of fruit (g)	28.65ns	35.63*	34.96*	36.21**	1.03

NOTE: **: Very Significant; *: Significant; ASO: Achichi Seed Oil; DTO: Days to Flowering; LSD: Least Significant Difference, WAT: Week After Transplanting.

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Conclusion

The study showed that the essential oils from Achichi seed (*Cannabis sativum* L.) are as effective as the Ridomil Gold ® synthetic fungicide in managing the bacteria spot of pepper. The treatments significantly boosted the growth and yields of pepper plants. The essential oil can therefore be incorporated in the bacteria spot management as an eco-friendly option to synthetic pesticides. This will lower the chemical residue levels in fruits of peppers thus improving the fruit quality and reducing the risks and hazards of toxic fungicides which aligns with sustainable agriculture. Also, ASO can be integrated into IPM strategies, lowering the dependence on single pest management approaches.

Recommendation

Further research should be conducted to determine the right volume between 2 – 4ml of ASO (achichi seed oil) extract that will effectively control or inhibit the severity of bacteria spot disease on pepper that will not lead to waste of material and resources.

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