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Survey and in vivo control of stem rot disease of groundnut using Allum sativum seed extracts in Adamawa State, Nigeria

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ABSTRACT
Groundnut fungal stem rot disease caused by Sclerotium rolfsii a destructive soil-borne fungal pathogen is one of the most importance disease of groundnut in Nigeria. The survey and control of stem rot disease of groundnut in Adamawa State was conducted from 2017 to 2023. The research focused on groundnut incidence, severity of stem rot diseaseand in vivomanagement of the disease pathogen using plant seed extracts of garlic (Allium sativum). Samples collected from nine local government areas of Adamawa State were taken to Plant Science laboratory of Modibbo Adama University, Yola in a dry sterile polythene bag. Laboratory work was carried out in the Department of Plant Science and Biotechnology, while field management of Sclerotium rolfsii was conducted in the Departmental farm, Federal Polytechnic Mubi. The result for incidence of stem rot disease of groundnut from the nine Local Government Areas of Adamawa State showed Mubi North had the highest incidence of 22.34 %, while Guyuk had the least incidence of 6.75 %. The level of stem rot disease severity revealed that Ganye recorded the highest severity of 4.60 and Guyuk had the least with 2.40. Plant extract materials were effective in inhibiting the growth of Sclerotium rolfsiiin vivo ($P \leq 0.05$). The level of inhibition increased with increase in concentrations but was not significantly different. High increase in growth (Number of Leaves =72.49 and Number of Branches =31.20) and yield characters (Number of Pod=44.47, Number of Matured Pod= 39.63 and Number of Healthy Pod= 36.30) were also recorded in the treated groundnut farms compared with the non treated control (Number of Leaves = 15.30, Number of Branches = 6.99, Number of Pod = 8.37, Number of Matured Pod = 2.20 and Number of Healthy Pod = 1.84). It is therefore recommended that the use of Allium sativumseed extracts for the management of groundnut stem rot should be encourage among local farmers at a concentration between 40-60 % because of its effectiveness.

Key words: Allium sativum, Sclerotium rolfsii,Stem rot, Pod yield and Arachis hypogea

I. INTRODUCTION

Groundnut (Arachis hypogea L.) is also known as peanuts, earthnuts, gobbers, pinders, manila nuts (Beghin et al., 2003). It is a member of the genus Arachis in the family Leguminosae (Fabacaea) which has replaced the traditional bambara groundnut (Vigna subterranean) in most countries of the world.The local names of groundnut include; English: Groundnut (Kumar et al., 2021), Spanish: Cacahuete (de Ron et al., 2019), French: Arachide (Kouaméet al., 2017), Portuguese: Amendoim (Lima et al., 2018), Swahili: Karanga (Mwai et al., 2016), Hindi: Mungphali (Singh et al., 2020), Bengali: Badam (Paul et al., 2018), Mandarin Chinese: Huasheng (Zhang et al., 2019), Japanese: Pīnattsu (Yamada et al., 2020) and Russian: Arakhis (Kozlov et al., 2017). In Nigeria local languages, Hausa: Gyada (Yakubu et al., 2019), Yoruba: Èpè (Adegbite et al., 2021), Igbo: Ùhòkè (Anyanwu et al., 2018), Efik: Akara (Udoh et al., 2017), Kanuri:

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Nyiori (Adamu et al., 2016). Groundnut originated from South America where the genus Arachis is widely distributed (Nagaveni et al., 2005). It is important seed and staple food commonly grown in Nigeria and in more than 100 countries in the world (ICRISAT, 2012). The major groundnut-producing countries include China, India, Nigeria, the United States, and Senegal (FAOSTAT, 2022). In Africa, groundnut cultivation is widely spread across various regions, including Western, Eastern, and Southern Africa (Fountain Publishers, 2014). As at September, 2021, Nigeria is the leading groundnut-producing country in Africa and one of the largest producers globally. It has favorable agro-climatic conditions for groundnut cultivation, and the country's production is supported by a large number of small-scale farmers and government initiatives (International Trade Centre (ITC), 2020).In Africa, where undernourishment from 2007–2008 increased by 10% with an increase in the price of nutritious foods, groundnut is an important cash crop, an affordable source of edible oil rich in omega-3 fatty acids, protein and vitamin E and its stover provides nutritious fodder for livestock (Pandey et al., 2012; Izgeet al.,2007; FAOSTAT, 2014). It provides high quality edible oil (48 to 50 %) (used in cooking, margarine, salads), easily digestible protein (26 to 28 %) and about half of the 13 essential vitamins and more than a 3rd (7) of the 20 essential minerals necessary for normal growth and maintenance (Tarawali&Quee, 2014).

Southern blight, also known as stem rot, is caused by a soilborne fungus. The disease is widespread on peanuts and other crops (Subrahmanyam et al., 2000). The fungus primarily attacks the base of stems near the soil line, but any plant part in contact with soil may be damaged. Infected plants are generally killed prior to maturity. Peg and pod infections are common and result in pod loss at harvest. The extremely broad host range of S. rolfsii also contributes to long-term survival between peanut crops (Farr and Rossman, 2014). S. rolfsii thrives in highly aerobic environments and thus survives best near the soil surface. The light-textured, slightly acid soils favored for peanut production also are very favorable for growth and survival of S. rolfsii. Complete resistance to southern stem rot is not known in cultivated peanut. However, a few cultivars with good partial resistance are available and are very useful for disease management (Chapin et al., 2010). In culture, mycelium appears smooth at first, but some cultures may develop aerial mycelia that cover all or part of the culture after a few days. The fungus produces at least two types of hypae, large diameter (5 to 9 µm) main branch hyphae and smaller diameter (2 to 4 µm) branch hyphae. (Shew, 2007). Populations of S. rolfsiiincrease in infested fields cropped to peanut unless control measures are taken (Subrahmanyam et al., 2000). High populations of the pathogen combined with favorable conditions for southern blight can result in yield losses of 25 percent or more.

The botanical bio-pesticides represent an alternative for pest control with low environmental impact and high food safety. Several products derived from plants have shown an antimicrobial effect. Among the main compounds present in these extracts are: flavonoids, phenols, terpenes, essential oils, alkaloids, lectins and polypeptides. Some plant extracts containing these metabolites has been extracted in water or other solvents, depending on its polarity, and in powder form (Bautista et al., 2003). Garlic is one of the famous natural products which were used since thousands of years ago as a vegetable, condiment and as a remedy; it was prescribed in many ancient civilizations such as Egyptian, Indian and Chinese civilizations (Jangam &Badole, 2014). Garlic (Allium sativum L.) is a bulb-shaped plant belonging to family Amaryllidaceae, there are about 300 varieties of garlic cultivated in many countries all over the world (FAO, 2007). Being an important food spice plant, it has significant role in disease prevention and control, many of the diseases can be cured with garlic (Yousuf et al., 2010). It has been used since long time against human pathogens. But studies are less regarding the usage of garlic against plant pathogens. Some earlier works (Kanan & Al-Najar, 2008; Obagwu& Korsten, 2003) deals with the action of garlic against pathogens. The flavor attributes which have unpleasant smell after consumption is related to its sulfur compounds which present in garlic in high quantity, recent scientific studies showed that these sulfur compounds are responsible for many medical benefits such as antimicrobial, anti-inflammatory, immunomodulatory, cardioprotective, antidiabetic, antioxidant, and anticancer activity (Martins et al., 2016).

II. METHODOLOGY

Study Area

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The study was carried out in the Botanical Garden and Laboratory of Department of Plant Science, Modibbo Adama University, Yola. Base on GPS coordinates, Adamawa State is located on Latitude 9^0 19' 60.00 "N and Longitude 12^0 29' 59.99" E (Google Map, 2023). It shares boundaries with Taraba State in the south and West, Gombe in its Northern Guinea Savanna ecological zone. The climate of the area is tropical with average temperature of 32°C and a relative humidity ranging from 15% to 68% (Chimatemps.com, 2015). The mean annual rainfall of Adamawa State ranges from 700mm in the North Western part to 1600mm in the Southern part; the length of the rainy season ranges from 120 – 210 days mostly distributed from May to October (Adebayo, 2004). The state relative humidity peak is usually in the months of August and September (Chama et al., 2007).

Sources of Groundnut Samples and Sample Size

Groundnut crop (whole plant) with stem rot symptoms was randomly collected from the three different farms of each Local Government Area (L.G.A.) selected among the geographical zones of Adamawa State (Mubi South, Mubi North, Michika from the Northern Senatorial zone, Song, Girei, Yola South from the central Senatorial zone and Ganye, Guyuk, Numan from the Southern Senatorial zone) as shown on Figure 1. Diseased groundnut crop was collected in a sterilized dry polythene bag and conveyed to the laboratory for laboratory analysis. A total of 270 samples were collected from nine (9) different Local Government Areas with 30 samples from each L.G.A (10 samples from each farm) using systematic sampling technique and was labeled according to the location. Three (3) farms were selected at random from each L.G.A at different locations from where samples were collected.

Collection of disease plant specimen

Incidence of groundnut stem rot on farm was determined. A quadrant of 3X3m was plotted out in each farm, and the stands were counted (healthy and diseased) samples. The samples collected from the farms were sampled out taking the number of diseased groundnut plants out of the total number of groundnut crops within the sample plot of each farm. The incidence of groundnut infection was expressed in percentage using the adopted formula given by Singh et al. (2012)

$$\frac{\text{Number of infected groundnut plants}}{\text{Total number of ground nut plants simpled}} \text{ X } 100\ \%$$

The severity of the disease on the infected plant was determined by using the visual scale of 1-5 in which:

1 = 1- 20 % of Groundnut Plants infected,
2 = 21- 40 % of Groundnut Plants infected,
3= 41- 60 % of Groundnut Plants infected,
4 = 61- 80 % of Groundnut Plants infected,
5= More than 80 % of Groundnut Plants infected.

The symptoms on the stem based on the 1-5 visual scales were grouped in the following categories based on the Ratanacherdchailet al. (2010) rating scale. Both the disease incidence and severity on the groundnut farm were compared. The data obtained from each farm was used to calculate and compare the averages for each LGA and subsequently average of local government area were used to estimate that of the state.

Medium for isolation and identification

The medium used for the isolation was Potato Dextrose Agar (PDA) (Zakawa et al. 2018). Thirty-nine (39) grams of PDA was dissolved into 1 litter of distilled water. The PDA was poured into conical flask, then covered with cotton wool and wrapped with aluminum foil before autoclaving it at 121^0 C for 15 minutes at 10 lbs pressure, and 200 hundred milligrams of chlorophenicol was added to the sterilized media, just before pouring into Petri-dishes to prevent bacterial growth and allowed to cool and solidify. The prepared media was autoclaved for 15minutes, 10Ib pressure and allowed to cool.

Isolation of the pathogen

The method of Burgess et al. (2008) was used. The diseased tissues (DT) from the periphery of the rotten groundnut stem were sectioned into $5mm^2$ pieces using sterilized scalpel after sterilizing the seeds in 0.1% mercuric chloride solution for 30 seconds and was rinsed in three changes of sterile distilled water. Sterilized pieces were picked with sterilized hot-flamed forceps, allowed to cool for a minute and were dried between sterile filter papers. With cold sterilized forceps, a sterilized piece of the infected part was then plated out on sterile solidified potato dextrose agar (PDA) and incubated at temperature of 30 ± 2^0C for 5 – 7 days and constant observation for any growth for sub-culturing. Pure isolates of fungal species were obtained by repeated

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sub-culturing on solidified sterile media and pure cultures were preserved in McCartney bottles containing solidified PDA in slants position. This was labeled according to organisms. The slants were corked loosely initially to enable the content fungus to grow and were then tightly corked and stored at a minimum temperature in a refrigerator to serve as stock cultures.

Identification of isolated fungus

Microscopic examination was made after examining the colony characteristics such as colony colour (front and reverse) and growth pattern and rate on media. A sterile needle was used to take a portion of the hyphae containing spores on to the glass slide which was stained with Lactophenol cotton blue and was observed under the light microscope with power objective lens X 40 for the structures of the fungi (Watanabe, 2010). Morphological structures such as septation of mycelia and nature of spores was also observed under the microscope and will be compared with the structures in Alexopouluset al. (2002).

Collection and preparation of plant extracts

The method of Ijatoet al. (2011) was used to prepare the ethanol extract. Fresh seeds of garlic plant were collected from Girei main market, Girei Local Government Area, Adamawa State. These were taken to the Plant Science Department of Modibbo Adama University, Yola.The collected plant seeds were rinsed thoroughly under running tap water and wereallowed to air dry under shade for 7 days. These were grounded, 80 g of the seed powder was dissolved in 100 ml of distilled water and shaken vigorously to give 80% concentration, likewise 60 g, 40 g and 20 g were dissolved into 100ml of distilled water each to give 60 %, 40 % and 20% concentration respectively in separate conical flasks and were kept for 24 hours. The sample was filtered with three layers' cheese cloth. The aqueous filtrate was used for control trials.

Land preparation

The land was cleared with cutlass, ploughed with tractor, harrowed and divided into ridges with a hoe. Field plot of 0.5 m X 0.4 m size with 0.5 m inter plot space, and 1.0 m outside border was used as adopted by Ibrahim and Dadari (2000). Groundnut seeds (Ordaaji variety) were sown with hoe within a space of 25 cm inter-row and 25 cm intra-row with a depth of 0.02m using the adopted method of Philip et al. (2010). The treatments consist of aqueous extracts of garlic seed, which consists of four sub-treatments i.e., concentration levels (20 %, 40 %, 60 % and 80 %). The experiment was laid out in a Randomized Complete Block Design (RCBD) and replicated three times. The plots were then infected with the fungal soil pathogen isolated from the laboratory and were watered for five (5) days before sowing of seeds.

Sowing

Sterilized healthy seeds of groundnut variety (Ordaaji) were selected and soaked with the extract at four different concentration levels according to the modified method of Idowu et al (2016) andAhmed et al. (2023). The dressed seeds were then sown at two seed per hole, at a spacing of 25 c m on row and 25 cm within row. The seedlings were later be thinned to one plant per hill at two weeks after planting. Weed control was carried out at the third and sixth weeks after planting using hoe to remove unwanted weeds. Remolding was carried out at 8-9 WAP to ensure proper weed control and a clean field at the time of harvesting.

Data collection

Data were collected on growth para meters(Germination Rate, Number of Leaves, Number of Branches, Length of Leaves, Branch Length), pathological characters(Leaves Defoliation, Flower Number of healthy pods and Number of unhealthy pods Abortion), yield parameters (Number of pods, Number of matured pods and Number of immature pods). Height, Number of Leaves, Number of Branches, Length of Leaves, Branch Length and Number of leaves per plant were taken after two weeks while numbers of matured and immature pods per plants were taken at harvest.

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III. RESULTS

Table 1: Incidence and Disease Severity of Groundnut Stem rot in Adamawa State

Locations	DI (%)	DS
Mubi North	22.34	4.40
Girei	17.32	3.20
Numan 12.23	3.00	
Michika 8.43	3.00	
Yola South	14.56	4.00
Song	9.10	2.60
Mubi south	7.54	3.00
Ganye	18.20	4.60
Guyuk 6.75	2.40	
LSD	1.23	1.10

Key: DI = Disease incidence
DS= Disease Severity

Table 2: Incidence and Disease Severity of Groundnut Stem Rot in Geopolitical Zones of Adamawa State

Geopolitical Zones	DI (%)	DS
NSZ	12.77	3.47
CSZ	13.67	3.27
SSZ	12.39	3.33
LSD (P≤0.05)	1.23	1.10

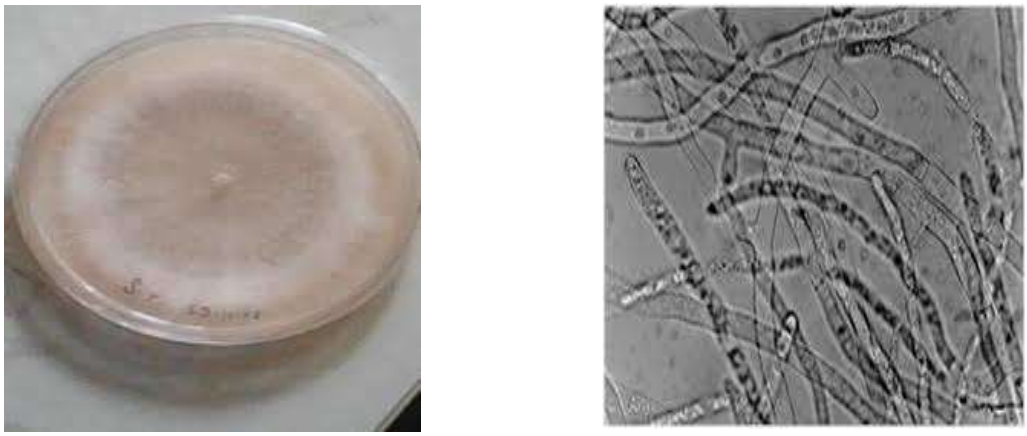

Plate I: (A) Four-day old pure culture Sclerotium rolfsii(B): Mircograph of Four Day Old Sclerotium rolfsii

Table 3: Effect of A. sativum Extracts on Growth Related Characters of Groundnut

Growth Characters	GC	BL	PH	NL	NB	LL
Extract	1.06	4.33	5.21	72.49	31.20	4.63
Control	0.97	1.52	0.42	15.30	6.99	1.99
LSD	0.07	0.42	0.51	5.28	1.99	0.41

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Key
GC= Germination Count
Bl= Branch Length
PH= Plant Height
NL= Number of Leaves
NB= Number of Branches
LL= Leaf Length

Table 4: Effect of A. sativumSeed Extracts on Pathological Characters of Groundnut

Plant Extracts	Leaf Defoliation	Flower Abortion
Seed	13.47	2.68
Control	2.14	0.81
LSD (0.05)	1.55	0.57

Table 5: Effect of A. sativumseed Extracts and Concentration on the Some Growth Characters of Groundnut Infected with Sclerotium rolfsii

Concentration(%)	Germination Count	No. of Leaves	No. of Branches
0	1.00	15.89	7.78
20	1.00	91.28	52.17
40	1.00	82.67	30.72
60	1.00	87.28	31.83
80	1.00	85.33	33.50
LSD (0.005)	0.07	0.42	0.51

Table 6: Effect of A. sativumseed Extracts and Concentration on the Pathological Characters of Groundnut Infected with Sclerotium rolfsii

Concentration (%)	Flower Abortion	Leaf Defoliation
0	2.61	0.94
20	14.44	3.61
40	17.39	3.32
60	17.33	2.61
80	14.56	3.00
LSD (0.005)	1.55	0.57

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Table 7: Effect of A. sativum Extracts on Yield Characters of Groundnut Infected with S. rolfsii

Extracts	Number of Pods	Number of Matured Pods	Number of Immature Pods	Number of Healthy Pods	Number of Diseased Pods
Seed	44.47	39.63	5.87	36.30	4.80
Control	8.37	2.20	6.17	1.84	6.53
LDS (0.05)	3.11	2.33	2.91	3.48	2.84

Table 8: Effect of A. sativum Extracts Concentrations on Yield Characters of S. rolfsii Infected Groundnut

Concentration (%)	Number of Pods	Number of Matured Pods	Number of Immature Pods	Number of Healthy Pods	Number of Diseased Pods
20	53.64	48.74	4.90	45.93	6.64
40	52.80	47.54	5.47	45.71	4.00
60	51.40	47.47	3.94	42.87	4.93
80	53.83	47.83	6.27	47.33	4.24
Control	8.37	2.20	6.17	1.84	6.53
LSD (0.05)	3.36	2.83	2.91	3.48	3.15

Plate II: Mixture of Matured and Immature Groundnut Pods, Diseased and Groundnut Pods Harvested from Experimental Farm as a result of infection by S. rolfsii

IV. DISCUSSION

Sclerotium rolfsii is the pathogen responsible for stem rot disease of groundnut in Adamawa State. The pathogen was also reported by Yan et al. (2021) to be the causative agent of stem rot disease of groundnut in Wuhan, Hubei, China. Genesanet al. (2007), Doley and Jite (2013) as wellas Leona et al. (2020) all reported this same pathogen (S. rolfsii) as the organism responsible for the stem rot disease of groundnut in their separate research conducted in India. Tarafdar et al. (2018) reported Sclerotium rolfsiias the major pathogen that reduces groundnut production by nearly 30 % asa result of stem rot disease caused by the pathogen. Sclerotium rolfsii is a destructive soil-borne fungal pathogen, it affects more than 600 plant species especially

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economically important agricultural and horticultural crops to include groundnut, soybeans, wheat, cotton, tomato, potato, cucurbit and onions (Yan et al., 2021). Sclerotium rolfsii can infect stems, root, pegs and pods of groundnut and cause branch wilting and even whole plant wilting. The pathogen produces white mycelium on infected plants and in culture, advancing mycelium and colonies often grow in a distinctive fan-shaped patternand coarse hyphal strands may have a somewhat ropy appearance. In agar plate culture, sclerotia are not form until the mycelium covers the plate. Sclerotia darken as they mature, becoming tan to dark brown in colour. Stem rot disease was recorded in all the local government areas visited during the survey and the virulence exhibited by the pathogen on groundnut seedling/plants were rated high.

Field management of groundnut plants showed that the garlic extracts materials were able to positively increase both the growth and yield parameters of groundnut plants. The plant height, number of leaves, number of branches and leaf shade of this research were better than that of the control. This agrees with Adeleke (2016) who reported that groundnut plants treated with the lower concentration of garlic extracts compared favorably with the control, while those with higher concentrations decrease in leaf area and plant height. Flower defoliation increases in treatments with garlic extract materials, this is however not in agreement with Koita et al. (2017) who reported that all plant extracts used controlled defoliation to a significant level compared to the negative control, which recorded the highest defoliation rate. In terms of yield, there was an increase in number of pods per plant, number of matured pods per plant and number of healthy pods per plant in all the plant treated with garlic extract materials. This agrees with Koita et al. (2017) that aqueous extracts from four plant species increased pod yield of infected groundnut over the negative control. Krishna and Pande (2005) reported that foliar application of Prosopis juliflora extract effectively reduced groundnut foliar disease severity and increased the pod yield. Another study revealed that foliar application of neem leaf extracts recorded significant improvement in pod yield and other yield characters of groundnut (Kumawat et al.,2009). Kongkaew and Phichai (2010) also found that dried garlic powder, which was extracted using a maceration method in distilled (DI) water and 95% ethanol solvent, was effective at inhibiting the growth of Trichoderma spp. isolated from Yanagi mushroom. Sittisartet al. (2017) reported that the dried leaves and fruits of garlic extracted using a Soxhlet extractor in DI water solvent where capable of preventing fungal infection in groundnut crop.

Several products derived from plants have shown an antimicrobial effect. Among the main compounds present in these extracts are: flavonoids, phenols, terpenes, essential oils, alkaloids, lectins and polypeptides. Some plant extracts containing these metabolites has been extracted in water or other solvents, depending on its polarity, and in powder form (Bautista et al., 2003). The enormous diversity of secondary metabolites and biological properties present in plants, are still subject of study. The limited knowledge that currently exists about plant extracts is an interesting point to begin studies with plants of almost any kind. Some families of plants may be more feasible for study, such as: Solanaceae for its high alkaloid content, or Mimosaceae that´s present species rich in tannins, or Lamiaceae and Meliaceae because of their wide diversity of terpenoids. For production of active ingredients, there are factors that determine variability in quality and quantity of metabolites. A plant may have different concentrations of a chemical in different vegetal parts: roots, leaves, flowers and fruit and may even be absent in one or more parts, so it is convenient to collect integral samples and also, knowing the chemical content of plants used in a given region, either as an insecticide, fungicide, nematicide, among others (Naqvi et al., 2011).

Recommendations

The use of garlic plant materials should be encouraged as they are effective in the management of the stem rot disease of groundnut.Further research should be conducted to evaluate the level of toxin contaminations caused by the isolate on infected groundnut seeds.

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