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Effect Of Plant Extracts Priming On The Control Of Seed-Borne Pathogenic Fungi Of Tomato (Solanum Lycopersicum L.) In Dadin-Kowa, Gombe State

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Abstract: Tomato is one of the important vegetable crops. The problem of seedling establishment is found in tomato due to several soil borne diseases. There are many chemical methods available to control these diseases but use of chemicals deplete the soil micro-environment and causes soil and water pollution and also do not fit within the framework of 'Organic farming'. Seed priming with certain phytochemicals may be an economic and ecofriendly alternative to such chemicals. A study was conducted in Federal College of Horticulture Dadinkowa, Gombe State to study the effect of tomato seed priming with plant extracts. The experiment was laid in a completely randomized design (CRD) replicated three times. In present study, two sources of tomato seeds were primed with extracts of three different plants (Neem seeds, ginger and Cassia alata leaves) and mancozeb which served as a positive check. Different leaf extracts doses of 40% was taken independently for seed priming in the laboratory and nursery conditions. It was found that priming with neem seeds, ginger and Cassia alata, extract had an improvement in seed germination, survival and seedling growth parameters in both conditions. Priming with mancozeb however was most promising in reducing the effect of pathogens in all the parameters. Seed priming with neem extract exhibited highest germination and survival rate (43.33% and 74.00%) and (48.70% and 46.70%) in farmers seed in laboratory and nursery respectively.

Keywords: germination; laboratory; nursery plant extracts; priming

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INTRODUCTION

Seed constitute the basic input for crop production in phanerogams so that pathogen free healthy seeds are considered as the vital factor for desired plant population and good economic harvest. Seeds are known to be contaminated with diverse fungal micro-propagules, some of them attack the seeds in the field internally contaminating the seed sheaths, tissues of embryo, endosperm and others under storage as a result of environmental conditions of high relative humidity, suitable temperature and high level of seed moisture content. The seed carrying organism's causes manifold loses to the crop and reduced the agricultural productivity (Bhajbhuje, 2013). Seed-borne diseases are able to spread across international borders very easily and are often difficult to identify as their typical symptoms being rare on seed surfaces as economic impact and importance has increased in recent years with regards to many kinds of crop worldwide (Lew-Smith, 2013). Planting infected seeds result in a widespread distribution of diseases within the crop, and an increased count of initial infection sites from which disease can spread. High rate of seed-to-seedling transmission of seed borne pathogens create alarming situation, even a small percentage of infected seed can result in significant seedling infection in the field (Saskatchewan, 2013). Major genera of plant-pathogenic seed borne fungi infect seedlings, some of them causing severe diseases. They limit the ability of plants to produce healthy fruit bearing shoots, causing damping-off, collar rot, stem canker, leaf blight and fruit rot resulting in premature defoliation, reduction in size and quality of fruits, thereby reducing potential yield to the extent of 20-30% (Lew-Smith, 2013). Seed deterioration is an inexorable, continuous and irreversible process, involves succession of seed borne fungal pathogens under storage resulting in loss of seed nutrients, alteration of physio-chemical properties of seeds, loss in seed weight, seed viability and vigour, medicinal properties, aesthetic changes including discoloration, heating and mustiness, cracking and abnormal odors contributing seed losses to the extent of 24% (Bhajbhuje, 1989). The consequences deterioration leading to series of deteriorative changes include membrane degradation, toxic metabolites accumulation, loss of enzymatic activity, lipid autoxidation, failure of repair mechanisms, genetic degradation, reduced productivity, finally loss of germinability or death of seed (Debnath *et al.*, 2012). Some fungal propagules may bring about certain biochemical changes and toxic metabolites that elicit a toxic response such as carcinogenicity, genotoxicity, terrotogenicity, hepatotoxicity, immunosuppression etc. Secondary fungal metabolites are reported to be toxic to man, animals and pose serious health hazard (Jain, 2008; Brakhage and Schroeckh, 2011; Shephard, 2012; Jyoti and Malik, 2013).

Tomato (*Solanum lycopersicum* L.) belongs to the family *solanaceae* and it is an annual sub-tropical fruit vegetable crop. The crop originated from South America and was introduced to Europe in the 16th Century and later to East Africa by colonial settlers in early 1900 (Wamache, 2005). In Nigeria, tomato plays a vital role in meeting domestic and nutritional food requirements, generation of income, foreign exchange earnings and creation of employment (Sigei *et al*., 2014). The crop is grown for both fresh domestic and export market but there is increasing demand for processed tomato products (Mungai *et al*., 2000).

Tomato crop does well in warm climate with an altitude range of 0 – 2100 m above sea level. It requires rainfall ranging between 760 mm to 1300 mm and deep fertile loam soil that is well drained, with high content of organic matter and a pH ranging between 5-7 (Rice *et al*., 1994). Fruits are used in salads or cooked as a vegetable, processed into tomato paste, sauce and puree. The nutritional value of tomato makes it a widely accepted vegetable by consumers. Fruits are rich in calcium, phosphorus, magnesium, copper, niacin, iron, folate, Vitamin A, B6, Vitamin E, Vitamin B2, Vitamin C, iron and carbohydrates (Wamache, 2005). Furthermore, the fruit has medicinal value as a gentle stimulant for kidneys and washing off toxins that contaminate the body systems. It improves the status of dietary anti-oxidants (lycopene, ascorbic acid and phenols) in diet (George *et al*., 2004). Tomato juice is known to be effective for intestinal and liver disorders (Wamache, 2005).

The cultivation of fruit and vegetables in Nigeria is undertaken by small farmers who usually have a small land holding of less than two hectares. As a result, the yield is low and coupled with inadequate postharvest experience, lack of storage facilities and postharvest diseases have made fresh tomato fruit unavailable abundantly all year round in the market in the country. The lack of postharvest management experience, sanitation of the environment of the farm and problem of handling and transportation may lead to pathogen infection which affects the quality of tomatoes. Large quantities of fruits and vegetables are produced and staggering yield figures are quoted as annual production. For example, 6 million tonnes of tomatoes was reported as the annual yield (Idah *et al*., 2007). However, it is the amount of the produce available to the consumer that is more important.

The major constraints to production in Nigeria are; pest and diseases, seed, rainfall, marketing, postharvest losses etc. Amongst the production constraints, pest and diseases are the major ones. The wilt diseases of tomato caused by *Alternaria solani* is one of the devastating diseases of tomato and can reduce yields of the crop significantly and above- all, largely seed transmitted. World-wide, there are numerous reports on seedborne fungi of tomato (Neegaard, 1977; Surayanarayana, 1978). Seedborne fungi are of considerable importance due to their influence on the over-all health, germination and final crop stand in the field. Farmers have to deal with

significant losses due to infections by serious seedborne pathogens on their plants, which may start from germinating seed, seedling in the nursery, matured plants in the field and proceed till the products are harvested and fruits and seeds stored. Significant crop losses due to seedborne pathogens have been recorded. Pimentel and Perkins (1980) for example, estimated total world food losses at about 45% due to diseases. The Commonwealth Agricultural Bureaux in their thirty-ninth annual report of 1968 estimated that losses due to diseases alone in the tropics are of the order of 10-13% (CAB, 1968).

Seed treatments for the control of soil-borne seedling diseases have generally met with failure. The only hope of controlling such diseases by seed treatment is that a sufficient quantity of fungicide be carried on the seed into the soil to protect the seedling until it becomes well established. Certain types of seed; such as -the tomato, will ·carry large quantities of material, but, when highly toxic compounds are used, there is almost always considerable damage done to the seed.

The control of seedborne pathogens is the first step in any agricultural crop production and protection programme. Attempts have been made to reduce seedborne infection by chemical treatment of the seeds and some successes have been reported. Messiaen (1992) reported that dressing seeds with non-systemic broad-spectrum fungicides such as Thiram, Maneb, Mancozeb, Difolatan, etc at rates of 2-4g a.i/kg of dry dust to very wrinkled hairy seeds of tomato and carrots can kill superficial fungi of spores of *Fusarium* species and *Alternaria* species and can protect seedlings before emergence against *Pythium* species. Though, chemical controls of seedborne pathogens have been very successful, however, chemical pesticides have the additional potential disadvantages of accumulation in the ecosystem and of induction of pesticide resistance in pathogens (Adeniji, 1970, Okigbo and Ikediugwu, 2000; Okigbo, 2004). There is also the problem of lack of expertise in the safe handling of chemical pesticides amongst most of the farmers. It is therefore, necessary to search for seed quality control measures that are cost effective, ecologically sound and environmentally safe to eliminate or reduce incidence of pathogens of economic importance to increase both seed germination and yield of plant crops.

In recent years much attention has been given to non-chemical systems for seed treatment to protect seeds against many plant pathogens (Nwachukwu and Umechuruba, 2001). Anti-fungal activity of different plant extracts has been reported earlier by several investigators against a number of plant pathogens (Hassan *et a*l, 2005; Yang and Clausa, 2007). However, information on management of seedborne fungal pathogens using botanicals on the major vegetable crops is generally lacking. There is therefore the need to investigate into the effect of different botanicals that will reduce or eliminate the incidence of plant pathogens and increase yields of crops.

The main objective of the study is to assess the presence and significance of pathogenic fungi on tomato seeds collected from the study area and the possibility of controlling these pathogens using botanicals.

MATERIALS AND METHODS

Experimental Area

The experiments were set out at the Pathology Laboratory and screen house, Federal College of Horticulture Dadinkowa, Gombe State.

3.1 Seed Health Testing

Tomato seeds were collected from tomato growers and commercial seed companies. Fifty out of the tomato seed samples collected were plated using the Blotter method at the Crop Protection Laboratory, Modibbo Adama University of Technology Yola, as recommended by Mathur and Kongsdal (2003). The petri-dishes with seeds were arranged in seed trays and incubated for 7 for 12 hours alternating cycles of light (near ultraviolet NUV or florescent daylight) and darkness to enhance sporulation of seedborne fungi. Each seed sample at the end of the incubation was examined thoroughly under stereomicroscope for the growth of fungi. Fungi found associated with seeds were carefully examined and identified based on 'habit characters' (Mathur and Kongsdal, 2003). Slide preparation of fruiting structures, such as conidia borne in conidiosphere, spores held together in spore masses, sporodochia, and acervuli, pycnidiospore in pycnidia, ascospores in perenthecia were examined each using compound microscope to confirm their identity using reference publication (Mathur and Kongsdal, 2003). Records were then taken on incidence and infection percent of the seedborne fungal pathogens identified on seeds.

Preparation of plant extracts

Fresh ring worm plant (*Cassia alata)* leaves and neem (*Azadirachta indica*) seeds were collected within the college environment, while ginger (*Zingiber officinale)* rhizomes and wettable mancozeb powder were purchased in Gombe main market and Agro-chemical stores respectively. Aqueous extracts of each of the plant materials were prepared as recommended by Okigbo and Nmeka (2005).

Neem seed extract

To prepare the solutions, eighty grams of de-pulped dry neem seeds, was weighed using the electronic weighing machine. The seeds were then ground in a blender with 200 ml distilled water. These were vigorously stirred and left to stand for one hour. The solutions were

later filtered through layers of muslin cloth. A concentration of 40% of the neem seed extracts was then prepared.

Ginger rhizome

Fresh ginger or *Zingiber officinale* rhizomes were washed and scrapped-off the outer-coverings/skins. Also 80 grams of the scrapped ginger rhizomes was weighed using the laboratory digital weighing machine. This was chopped into pieces and separately ground in a blender with 200 ml of distilled water. It was vigorously stirred and left to stand for one hour. The solutions were filtered through muslin cloth and concentration of 40% of ginger rhizome extracts was prepared.

***Cassia alata* extracts**

Fresh leaves of *Cassia alata* were washed in the laboratory with tap water; 80 g of the washed fresh leaves was weighed using the laboratory weighing machine. The leaves were ground in a blender with 200 ml of distilled water. These were vigorously stirred and left to stand for one hour. The solutions were filtered through muslin cloth. Concentrations of 40% of *Cassia alata* extracts were prepared.

Seed treatment using plant extracts

Samples of the infected tomato seeds were treated with each of the plant extracts by soaking the seeds in each of the concentrations for 12 hours. Treated seeds were dried on clean sheets of paper overnight under room conditions. Seeds were also soaked in 3% concentrated aqueous Mancozeb solutions for 12-hour period and dried under the same conditions similar to the other treatments, while untreated seeds (seeds soaked in distilled water) serving as the control.

Determination of the effects of plant extracts on percent incidence of fungal pathogens and seed germination

Three replicates of 50 seeds per Petri-dish for each of the treated tomato seeds including the controls were plated using the Blotter Method as recommended by Mathur and Kongsdal (2003). They were then observed for 10 days and then examined for seedborne pathogens. Records on incidence of seedborne fungi and germination of treated seeds were taken.

Preparation of plant growing media

Top-soil was steam pasteurized at the Horticulture Department of the Federal College of Horticulture Dadinkowa. Seed sowing pans filled with sowing mix made up with the pasteurized top-soil and fine river sand at the ratio of 2:1 was arranged randomly in the Screen House.

Sowing of the treated tomato seeds

The trial was arranged in a Completely Randomized Design (CRD). For each of the treatments, three replicates of 50 seeds were sown making a total of 250 seeds for each treatment. Sowing was done by the broadcast method and fine sieved pasteurized top-soil was spread evenly on seeds before watering using a watering can and a fine netted screen was used to cover all the bowls containing the treated sown tomato seeds. Watering of germinating seedlings was done every other day with equal amounts of water in the screen house. Records on germination, seedling mortality, and seedling population were taken at 30 days after sowing.

Growing tomato seedling under fine netted screen

Parameters studied
Seed health testing

Data on the incidence and severity of pathogenic fungal infection was collected by examination of incubated seeds under stereomicroscope and compound microscope as recommended by Mathur and Kongsdal (2003).

Determination of effect of plant extracts on incidence of seedborne fungi

The Blotter Method of Mathur and Kongsdal (2003) were used to determine the effect of plant extracts on incidence of important fungal pathogens. This involved plating treated seeds, i.e. 3 replicates of the 5 treatments at the concentration levels of the plant extracts and the recommended Mancozeb (fungicide) and the controls. Observations for the incidence of important fungal pathogens were made under microscope at the end of the incubation period. Records on incidence will be taken using the standard recording sheets.

***Effects of plant extracts on percent seed germination (Laboratory* experiment)**

Records on percent germination were made through counting of normal, abnormal seedlings, freshly un-germinated, hard and dead seeds per treatment in the Laboratory.

Effects of plant extracts on percent seed germination (Screen house experiment)

Standard germination test

Three replications of 50 seeds of each treatment were counted and sown in free drainage plastic bowls of 35cm diameter filled with the sowing-mix' mixed up with the sterilized top-soil and a fine river sand in the ratio of 2:1. Germinated seedlings were counted at 14 days after sowing. Seeds were considered germinated when the radical has emerged from the seed coat with the cotyledons partly or fully exposed.

Effect of plant extracts on seedling mortality and seedling population

The method of Daftari and Verma (2006) was used to determine the effect of plant extracts on seedling mortality and seedling population per treatment. This involved the count of dead and dying seedlings due to infections by seedborne fungi associated with the seed for seedling mortality, while seedling population per treatment were determined by counting the number of surviving healthy seedlings. Records were then taken on seedling mortality and seedling population per treatment at 3 and 4 weeks after sowing respectively.

Statistical analysis of Data

Analysis of variance (ANOVA) was performed on all the data collected in respect of parameters studied on effects of plant extracts and separation of treatment means was done using the LSD at 5% level of significance.

RESULTS AND DISCUSSION

Incidence of fungal species on tomato seeds

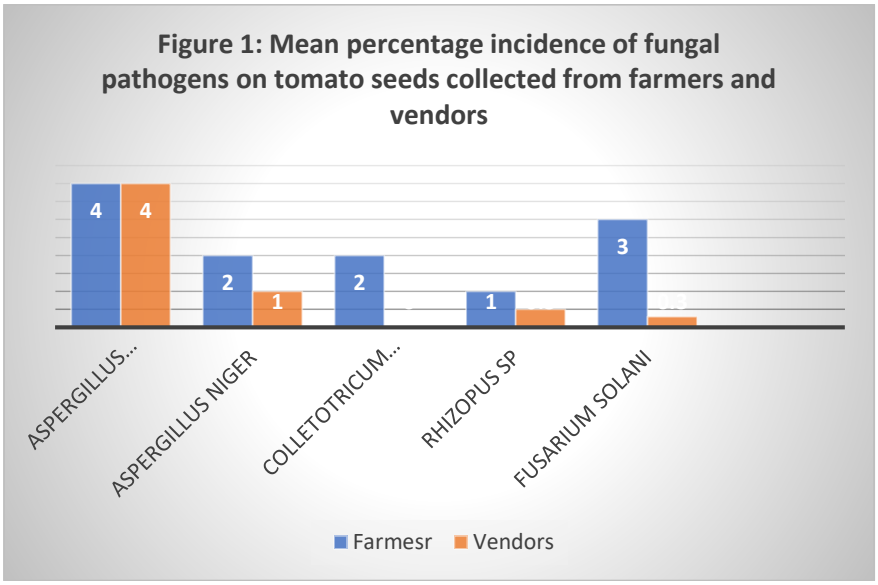
Out of the fifty seed samples tested for seedborne fungal pathogens, a total of 4 genera of 6 species of fungi were recorded (Figure 1). The mean percentage incidence and percent severity of seedborne fungi of tomato revealed by the Blotter Method are given.

The fungal pathogens recorded were *Aspergillus flavus, Aspergillus niger, Colletotrichum dematium, Penicillium spp, Rhizopus spp* and *Fusarium solani.*

The Blotter Method results revealed that apart from *Aspergillus flavus, A. niger, Penicillium spp and Fusarium solani* all other fungal pathogens were recorded in the farmers with a mean incidence of 2.00 percent. The storage fungus (*Aspergillus flavus*) recorded the highest mean infection percentage of 4 percent, while the lowest mean infection of 0.3 percent was recorded by *Fusarium Fusarium solani.*. In the seeds collected from vendors, apart from *Rhizopus spp and Colletotricum dematium* all other fungal pathogens were recorded with a mean incidence of 0.92 percent. *Aspergillus flavus* recorded the highest mean infection of 4 percent, while *Fusarium solani* recorded the lowest mean infection of 0.3 percent.

The highest percentage fungi incidence as well as severity on tomato seeds collected from different farmers may be attributed to inadequate seed drying by most tomato farmers. It may also be attributed to their storage environment (i.e. under high storage humidity and temperatures). In contrast, the lowest incidence of fungal pathogens recorded from vendors' seeds may be attributed to the proper drying of extracted tomato seeds before storage. Of the fungal species identified, *Fusarium spp* and the storage fungi (saprophytes), for example *Aspergillus spp* were found to be predominant. Similar reports have been reported by Orlova *et al*. (1982), Marcinkowska (1982) and Huang and Sun (1986) who found that *Alternaria alternata, Aspergillus flavus, Aspergillus niger* and *Fusarium oxysporum* were predominantly associated with tomato seeds.

Aspergillus, Rhizopus and *Penicillium* sp. are probably not pathogenic pathogens on tomato. They are however, storage fungi (saprophytes). Harman and Pfleger (1974) and Kulik (1973) reported that *Aspergillus* sp. have no effect on germination of tomato seeds. Of the high incidence of storage fungi, these conditions were found to be as a result of their pre-treatment of their seeds and farmers' storage condition of their seeds.



Effect of plant extracts on percentage seed germination of tomato in the screen house

The results of the effects of plant extracts on percentage seed germination of tomato in the laboratory and screen house is presented in Table 1. The result shows highly significant (P<0.05) differences among the extracts for seeds collected from farmers. Results of the study for in-vivo tomato seed was almost similar as obtained by the in-vitro seed germination test. Kuhn and Hargreaves (1997) reported that substances found fungicidal in-vitro, in almost all cases kill the fungus in-vivo and could improve upon subsequent seedling growth when used properly in the seed treatment. In the comparison for example, seed treated with mancozeb recorded the highest percentage germination of 77.30 percent just like the in-vitro experiment with the highest percent (83.30%). Reasons for the high percentage germination by mancozeb treatment may be attributed to the suppression of fungi incidence and above – all, offered a better protection of embryos of the treated tomato seed from damage by *Fusarium moniliforme*.

Table 1: Effect of plant extracts on percentage seed germination of tomato in the laboratory and nursery

Treatment	Laboratory		Nursery	
	Farmers Seed	Vendors Seed	Farmers Seed	Vendors Seed
Mancozeb	59.33	77.30	77.30	83.30
Neem Seed	43.33	64.00	74.00	54.70
Ginger	40.00	68.70	62.00	64.70
Cassia	36.00	62.00	66.70	80.00
Control	32.67	38.70	38.70	60.30
P<F	<0.001	<0.001	<0.001	0.019
LSD (0.05)	6.904	11.850	12.810	16.700

Effect of plant extracts on percentage seedling mortality of tomato

The result for the effect of plant extracts on percent seed mortality of tomato is presented in Table 2. Results of the study on seedlings mortality indicated significant differences (P<0.05) for the effects between plant extracts. Significant reductions of tomato seedlings were therefore observed. Mancozeb achieved the highest of seedling mortalities both in the laboratory and in the screen house. One of the reasons being that, earlier results indicated that mancozeb had some inhibitory effects and that probably could have accounted for the significant reductions. Similarly, results of the study also indicated that extracts of neem seeds compared favourably to mancozeb to achieve control in tomato seed. The reasons may be due to its greater quantities of antifungal activities and that could have contributed in achieving a better control than the other plant extracts. Infected seed is less viable, has low germination, reduced vigour and reduced yield (van Gastel, 1996). Wilting and death of shoot in cucurbits caused by *Fusarium moniliforme* has been reported by Palodhi and Sen (1980).

Table 2: Effect of plant extracts on seedling mortality of tomato in laboratory and nursery conditions

Treatment	Laboratory		Nursery	
	Farmers Seed	Vendors Seed	Farmers Seed	Vendors Seed
Mancozeb	12.70	18.70	24.00	27.37
Neem Seed	14.00	19.30	25.30	33.30
Ginger	16.70	23.30	24.00	30.30
Cassia	13.30	27.30	32.00	33.30
Control	45.30	50.00	44.70	50.70
P<F	0.002	0.007	0.004	0.005
LSD (0.05)	6.50	14.310	10.880	10.630

Effect of plant extracts on seedling population (%) of tomato

Results of the study revealed that there were significant differences (P<0.05) for the effects between plant extracts for both laboratory and screen house experiments. The results of the study showed that mancozeb gave the highest seedling population (39.30% and 58.70%) in the laboratory and (52.70% and 58.70%) in the screen house. It is possible that mancozeb might have possessed greater and stronger anti-fungal activities that probably remained with the seedlings throughout the growth period to offer better protection against seedling mortality. Secondly, seeds treated with mancozeb gave the same good percentage germination at the end of the study period. Thirdly, mancozeb did not encounter serious seedling mortality incidence unlike *Cassia alata, Zingiber officinale* and *Azadirachta indica* extracts which recorded significant percentage seedling mortality. This work is in agreement with the findings reported by Sinnadurai, (1992) that optimum plant densities can influence increased yield and one of several means of achieving this is through the use of good quality seed (Asuboah, 2009). Quality seeds can also be obtained through the use of effective seed treatment method (Asuboah, 2009).

Table 3: Effect of plant extracts on seedling population (%) of tomato in the laboratory and nursery conditions

Treatment	Laboratory		Nursery	
	Farmers Seed	Vendors Seed	Farmers Seed	Vendors Seed
Mancozeb	39.30	58.70	52.70	58.70
Neem Seed	29.30	44.70	48.70	46.70
Ginger	22.70	45.30	42.70	37.30
Cassia	22.70	34.70	30.00	34.70
Control	7.30	22.00	20.70	22.00
P<F	0.019	<0.001	0.002	<0.001
LSD (0.05)	9.250	16.650	9.810	14.400

CONCLUSION

It can be concluded from this work that the use of neem seed extract has the potential of controlling the pathogenic fungi of tomato seeds. Generally, the results also indicated that all plant extracts used to treat the seeds did not significantly reduce seed germination and plant mortality of tomato. However, mancozeb was comparably most efficacious in the control of pathogenic fungi. Tomato farmers should therefore use neem seed extract for seed priming.

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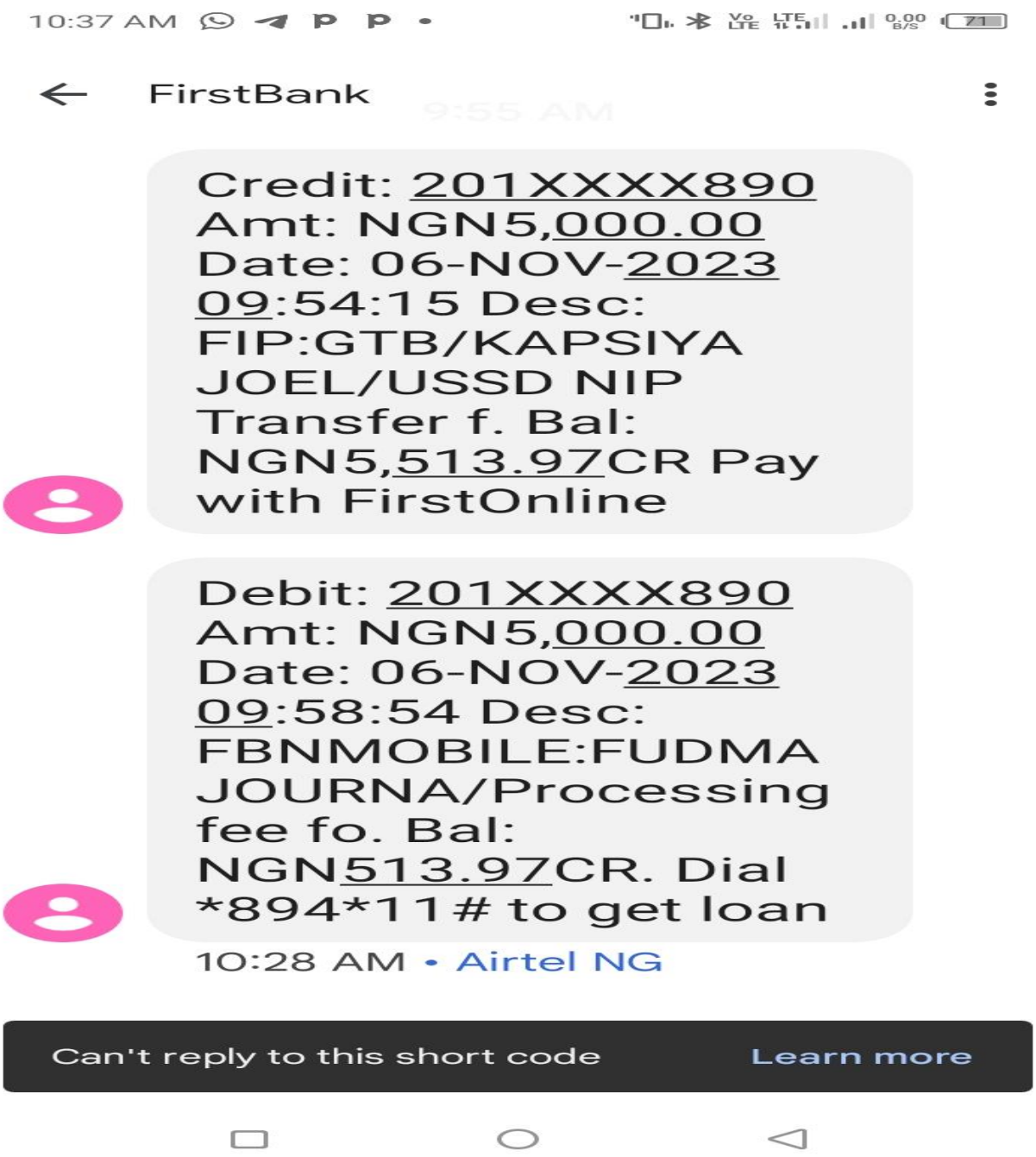
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Transfer f. Bal:
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with FirstOnline
Debit: 201XXXX890
Amt: NGN5,000.00
Date: 06-NOV-2023
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JOURNA/Processing
fee fo. Bal:
NGN513.97CR. Dial
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