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ORIGINAL RESEARCH ARTICLE

Field evaluation of single and interaction effects of nematode, fungus and bacterium on nodulation and pathological parameters of Bambara groundnut (*Vigna subterrenea* (l.) Verdc.) in field condition

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ABSTRACT

The aim of the present study was to determine the effects of single and mixed infections of nematode (*Meloidogyne javanica*), fungus (*Fusarium oxysporum*) and bacterium (*Xanthomonas axonopodis*) on nodulation and pathological parameters of Bambara groundnut (*Vigna subterrenea* (L.) Verdc.) in field condition. Nematode infested field was used while other pathogens were obtained from diseased plants. The Randomized Complete Block Design (RCBD) was adopted in a 5 × 9 × 5 factorial design (5 blocks, 9 treatments and 5 replicates per treatments) resulting in 225 experimental units. In each experimental unit, three seeds were sown to a depth of 5cm and thinned to one plant per planting hole after germination at day 7. Treatments were inoculated into test plant following standard methods. As a result, the control treatment recorded the highest number of nodules (64.0 ± 6.91), followed by bacterium (45.2 ± 5.11) while N + F + B had the lowest number of root nodules (23.4 ± 2.42). Simultaneous treatment (N + F + B) gave the highest percentage reduction in nodulation (63.44%), followed by treatment N + F7 (56.25%). Fungus treatment recorded the highest mean wilted plants (3.8 + 0.20) followed by N + F7 treatment (3.40 + 0.40). Gall formation in the nematode treatment increased proportionately by 56.33% as the highest recorded, followed by treatment N + F7 with 50.0%. Treatment N + F7 had the highest reproduction factor (Rf) value of 9.30 followed by nematode (8.30), N + B7 (7.40), N + F + B (6.80) and N + F14 (6.50). Zero (0) Rf value was recorded in fungus, bacterium and control treatments. The observed differences in nodulation and pathological parameters among the treatments are significant ($P < 0.05$). The data provided in this work is important in the control of the three pathogens affecting the productivity of Bambara nut. Formulation of a single protectant should be designed to have potent effects on the three pathogens to achieve effective protection and good production of Bambara nut.

Keywords: mixed infection; Bambara; nodulation; pathology; productivity; protection

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1. Introduction

Bambara groundnut (*Vigna subterrenea* (L.) Verdc.) is an African legume whose cultivation predates that of groundnut[1]. The distribution of wild Bambara groundnut is known to extend from Jos-Plateau and Yola-Adamawa in Nigeria to Garouna in Cameroon[2,3]. It is now widely distributed in the semi-arid zone of Sub-Saharan Africa (SSA)[4]. Bambara groundnut plays a key role in the traditional food and culture of West Africa. It is the third most important food legume after cowpea and groundnut[5,6]. Benue State, the dominant tribes (Tiv, Idoma, igede and the Etulo) are engaged in intensive production of Bambara nut mainly for the production of locally made tasty bean product known as “okpa”.

The crop's productivity is affected by diseases caused by nematodes, fungu, bacteria and viruses. The root knot nematode; *Meloidogyne javanica* has been reported to cause losses ranging from 15% to 60% in vegetables such as okra, brinjal, French beans, cowpea, and soybeans[7–9]. Root knot nematodes causes poor root development resulting in reduced nutrient and water uptake, in addition to weak support for the plant as well as significant changes in the root tissues, development of prominent feeding sites called giant cells (GCs)[9]. Among the fungal diseases of Bambara groundnut, Fusarium wilt caused by *Fusarium oxysporum* (Schlect) Synder and Hansen, is a limiting factor in Bambara production and yield[1]. This soil-borne pathogen is highly specific to vegetables and legumes and is worldwide in distribution[9]. Bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* is a major biological constraint to increase cowpea production in small holder farming sector and is prevalent in major cowpea growing areas of the world[10]. Siddiqui *et al*.[11] observed that wounds created by nematodes apparently favours bacteria more than fungi. Interaction effects of pathogens on the growth have been reported in other legumes[6,9].

It is important to understand the nature and effects of single and mixed interactions of three important pathogens limiting the productivity of Bambara groundnut in Nigeria. Hence, the present study was undertaken to determine the effects of single and mixed infections of nematode (*Meloidogyne javanica*), fungus (*Fusarium oxysporum*) and bacterium (*Xanthomonas axonopodis*) on nodulation and pathological parameters in Bambara groundnut (*Vigna subterrenea* (L.) Verdc.) in field condition.

2. Materials and methods

2.1. Experimental site

The field experiment was located at the Agronomy Research Farm, Federal University of Agriculture Makurdi, Benue State (now known as Joseph Sarwuan Tarka University Makurdi), tropical Guinea Savanna (7°43′50″ N and 8°32′10″ E). The annual average rainfall was 1090 mm with a temperature range of 27.8 °C–28.2 °C minimum 30.1 °C–34.1 °C maximum[7]. The field used this investigation had previously been cropped to tomato and egg plants with high *Meloidogyne javanica* infestation but had no incidence of *Fusarium oxysporium* or *Xanthomonas* spp. infection[7].

2.2. Collection of Bambara nut seeds

Healthy seeds of Bambara groundnut (SUAN variety) were sourced from the seed store of the Department of Plant Breeding and Seed Science, Federal University of Agriculture Makurdi, Benue State.

2.3. Sterilization of seeds, glass ware and soil

Seeds were surface sterilized using 1.08% Sodium hypochlorite solution[12]. Other materials were either air dried as in plastics or oven dried as in glass wares or autoclaved as in media. All laboratory activities were carried out under aseptic condition[7].

2.4. Sources of test pathogens

Fusarium oxysporum was isolated from stem and root of diseased okra (*Abelmoschus esculentus* L.) using standard methods[7,13] and sub-cultured on Potato Dextrose Agar (PDA) plates[14] followed by morphological identification on the stereobinocular microscope and the use of identification guide[15]. Spores were separated from the mycelium to obtain fungal inoculum. *Xanthomonas axonopodis* was isolated from diseased cowpea plant in the field[10], cultured on the Nutrient agar and sub-cultured on the YPSA (Yeast Peptone Sucrose Agar)[16]. Exactly 10ml of the suspension containing 1×10^{-6} of the bacteria was used to inoculate the test plants[11]. *Meloidogyne javanica* was collected from galled roots of infected tomato plants (*Solanum lycospersicum* L.) in the field using standard methods[12]. The infected roots were examined for the presence

of galls containing adult female and juvenile[17,18] using perineal pattern morphology as compared with standard pictorial guide[19].

2.5. Pre-planting estimation of nematode population

Nematode population (second stage juveniles; J2) was estimated before treating and sowing/planting through random collection of soil samples to a depth of 20 cm using a soil auger[7]. Bulked soil was used for nematode extraction, sieving and decanted with Baermann's funnel[13]. The number of nematodes was counted under a Stereomicroscopic microscope at ×40 magnification. Counting was done thrice and the average number of nematodes was obtained.

2.6. Field experimental design and seed sowing

The Randomized Complete Block Design (RCBD) was adopted. It consisted of five blocks each measuring 13 m × 2 m spaced 1 m from each other[18]. The experiment was a 5 × 9 × 5 factorial design (5 blocks, 9 treatments (T1–T9), 5 replicates per treatments) resulting in 225 experimental units in each experimental unit, three seed of Bambara groundnut were sown to a depth of 5 cm and thinned to one plant per planting hole after germination at day 7[18]. The treatment combinations are described in **Table 1**.

Table 1. Treatment combination and description.

S/N	Treatments	Description	Symbol/code
1	T1	Seedlings exposed to natural infection by the nematode in the soil at planting.	N
2	T2	Seedlings inoculated with 10^{-6} cfu/mL of fungus alone (F) fter sowing in Carbofuran (Furadan 10 g) treated soil.	
3	T3	Seedlings inoculated with 10 mL of bacterial suspension (10^{-6} cfu/mL) alone (B) in Carbofuran (Furadan 10 g) treated soil.	B
4	T4	Seedlings exposed to natural infection by nematode in the soil followed by 10^{-6} cfu/mL of fungus and 10 mL of bacterial suspension simultaneously.	N + F + B
5	T5	Seedlings exposed to natural infection by nematode in the soil followed by inoculation with 10^{-6} cfu/mL of fungus 7 days after germination.	N + F7
6	T6	Seedlings exposed to natural infection by the nematode in the soil followed by inoculation with 10 mL of bacterial suspension 7 days after germination.	N + B7
7	T7	Seedlings exposed to natural infection by the nematode in the soil followed by inoculation with 10^{-6} cfu/mL of fungus 14 days after germination.	N + F14
8	T8	Seedlings exposed to natural infection by nematode in the soil followed by inoculation with 10 mL of bacterial suspension 14 days after germination.	N + B14
9	T9	Seedlings with no nematode, fungus or bacterium in plots treated with Carbofuran (Furadan 10 g) which served as control (C).	C

Note: Carbofuran is a broad spectrum carbamate insecticide and nematicide used as a positive control.

2.7. Inoculation of test plants in field experiment

Inoculation of test plants with fungus and bacterium was done 7 and 14 days after germination (DAG), whereas nematode infection was by naturally occurring nematode population in the soil. Inoculation of test plants was done following standard methods[20].

2.8. Estimation of nematode population at harvest

The number of second stage juveniles of nematode per treatment was determined by carefully mixing the soil in each treatment with a hand trowel. Then, nematodes from a 250 cm soil sub-sample from each treatment was extracted by combining Cobb's sieving and decanting technique with the Baermann's funnel method[13]. Similarly, eggs and second stage juveniles of the nematode in 5 g of galled roots were extracted following the methods described above. Nematode suspension was collected and the number of juveniles was counted under the stereoscopic microscope at ×40 magnification[7].

2.9. Estimation of wilt incidence

The number of wilted plants was counted and recorded. Percentage wilt incidence was calculated as described by Nene et al.[21].

$$\text{Percentage wilting (\%)} = \frac{\text{Number of wilted plants} \times 100}{\text{Total number of plants}}$$

2.10. Estimation of root galls (RG)

Each plant was carefully uprooted and washed under tap water to free the roots from adhering soil particles. Roots were examined for galls and the number of galls counted[22]. Mean gall root was computed for various treatments.

2.11. Nematode reproduction factor (RF)

Nematode reproduction factor was determined following the method of Afolami et al.[23].

$$\mathbf{Rf} = \frac{Pf}{Pi}$$

where Rf is nematode reproduction factor; Pf is the final population of nematode from roots and soil; Pi is initial population of nematode (200 J2).

3. Results

3.1. Effects of pathogens on plant nodulation

Table 2 shows the single and mixed effects of pathogens on the number of nodules produced by Bambara plants in the field. Control treatment recorded the highest number of nodules (64.0 ± 6.91), followed by bacterium (45.2 ± 5.11) while N + F + B had the lowest number of root nodules (23.4 ± 2.42). The observed differences are significant among the treatments (F = 19.29, $P < 0.05$) and blocks (F = 9.02, $P < 0.05$). The following treatments showed significant effects: control, bacterium, N + F + B, N + F7 and N + B14 ($P < 0.05$). **Table 3** gives the average number of nodules per plant and the percentage reduction in nodules caused by inoculated pathogenic treatments when compared with the control in field. The control treatment gave the highest mean number of nodules of 12.8 ± 2.56 per plant while treatment N + F + B gave the lowest mean number of nodules per plant (4.68 ± 0.48). As a result, simultaneous treatment N + F + B gave the highest percentage reduction in nodulation (63.44%), followed by treatment N + F7 (56.25%), while inoculation wit bacterium recorded the least (26.38%).

Table 2. Field evaluation of single and mixed infection on number of nodules.

Treatments/Blocks	Total B1	Total B2	Total B3	Total B4	Total B5	Grand mean TRT
N	50	35	32	29	26	34.4 ± 4.8^{bcd}
F	37	37	34	30	27	33.0 ± 1.97^{bcd}
B	65	44	42	38	37	45.2 ± 5.11^{b}
N + F + B	31	25	22	23	16	23.4 ± 2.42^{d}
N + F7	36	27	30	26	21	28.0 ± 2.47^{cd}
N + B7	60	28	34	31	26	35.8 ± 6.2^{bcd}
N + F14	41	40	33	30	20	32.8 ± 3.81^{bcd}
N + B14	71	39	35	33	27	41.0 ± 7.75^{bc}
C	88	60	64	63	45	64.0 ± 6.91^{a}
Mean block	53.22 ± 6.37^{a}	37.22 ± 3.57^{b}	36.22 ± 3.88^{b}	33.67 ± 3.92^{b}	27.22 ± 2.96^{c}	

* Means that do not share same letter are significantly different.
F (Treatment) = 19.29, $P = 0.000$ ($P < 0.05$)
F (Block) = 9.02, $P = 0.000$ ($P < 0.05$)

Table 3. Effect of single and mixed infection on average number of nodules per plant.

Treatments	Ground mean TRT in all block	Average number of leaves per plant	% reduction in number of leaves against control
N	34.4 ± 4.8^{bcd}	6.88 ± 0.96	46.25
F	33.0 ± 1.97^{bcd}	6.60 ± 0.39	48.44
B	45.2 ± 5.11^{b}	9.04 ± 1.02	26.38
N + F + B	23.4 ± 2.42^{d}	4.68 ± 0.48	63.44
N + F7	28.0 ± 2.47^{cd}	5.60 ± 0.49	56.25
N + B7	35.8 ± 6.2^{bcd}	7.16 ± 1.24	44.06
N + F14	32.8 ± 3.81^{bcd}	6.56 ± 0.76	48.75
N + B14	41.0 ± 7.75^{bc}	8.20 ± 1.55	35.94
C	64.0 ± 6.91^{a}	12.8 ± 2.56	

* Means that do not share same letter are significantly different at $P \leq 0.05$ using Fisher's LSD.
* Values are means of five replicates.
Friedman S (Treatment) = 13.11, $P = 0.015$ ($P < 0.05$)

3.2. Effects of pathogens on nematode population

Table 4 presents the effects of treatments on nematode population. The highest mean value (930 ± 97.0) was recorded in N + F7 treatment and it was followed by 830 ± 81.5 in single treatment with nematode (N) while the lowest population of 30 ± 20.00 was found in the control treatment. Inoculation with bacterium or fungus gave zero (0) nematode population. Significant differences were recorded in the mean nematode population of all the treatments.

Table 4. Field evaluation of single and mixed effect of pathogens on nematode population in the root and soil.

Treatments/Blocks	Total B1	Total B2	Total B3	Total B4	Total B5	Grand mean TRT
N	850	1050	950	700	600	830 ± 81.5^{ab}
F	0	0	0	0	0	0.00 ± 0.00^{c}
B	0	0	0	0	0	0.00 ± 0.00^{c}
N + F + B	550	800	650	700	700	680 ± 40.6^{b}
N + F7	950	1200	1000	900	600	930 ± 97.0^{a}
N + B7	550	900	800	800	650	740 ± 62.0^{ab}
N + F14	700	750	700	600	500	650 ± 44.7^{b}
N + B14	600	650	650	500	700	620 ± 33.9^{b}
C	0	100	50	0	0	30 ± 20.00^{c}

* Means that do not share same letter are significantly different at $P \leq 0.05$ using Fisher's LSD.
* Values are means of five replicates.
Friedman S (Treatment) = 11.29, $P = 0.024$ ($P < 0.05$)

3.3. Effects of pathogens on plant wilting

Table 5 shows the effects of treatments on number of wilted plants. Fungus treatment recorded the highest mean wilted plants (3.8 + 0.20) followed by N + F7 treatment (3.40 + 0.40) while the least was recorded in N + B7 treatment (1.80 + 0.37) while bacterium and control treatments had no wilted plants.

Table 5. Field evaluation of single and mixed infection effect of treatment on number of wilted plants.

Treatments/Blocks	Total B1	Total B2	Total B3	Total B4	Total B5	Grand mean TRT
N	3	3	2	1	2	2.2 ± 0.37^{bc}
F	4	4	4	4	3	3.8 ± 0.20^{a}
B	0	0	0	0	0	0.00 ± 0.00^{d}
N + F + B	3	4	3	2	2	2.80 ± 0.37^{abc}
N + F7	4	4	4	3	2	3.40 ± 0.40^{ab}
N + B7	3	2	2	1	1	1.80 ± 0.37^{c}
N + F14	3	3	2	2	1	2.20 ± 0.37^{bc}
N + B14	2	3	2	1	2	2.00 ± 0.32^{bc}
C	0	0	0	0	0	0.00 ± 0.00^{d}
Mean block	2.44 ± 0.50	2.56 ± 0.53	2.11 ± 0.48	1.56 ± 0.44	1.44 ± 0.34	

* Means that do not share same letter are significantly different at $P \leq 0.05$ using Fisher's LSD.
Friedman S (Treatment) = 12.73, $P = 0.013$ ($P < 0.05$)

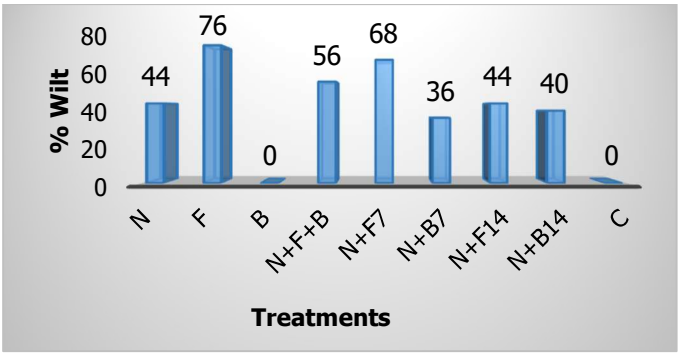

Figure 1. Single and mixed effects of pathogens on percentage wilt in Bambara field condition.

Significant difference exists among the treatments (F = 12.73, $P < 0.05$). However, the effects of N, N + F14 and N + B14 treatments were the same ($P > 0.05$). Result on percentage wilt (**Figure 1**) revealed that treatment fungus treatment had the highest percent wilt (76%), followed by N + F7 (68.0%) and N + F + B (56.0%) treatments while the least was recorded in N + B14 (40.0%). 0% wilt was recorded in bacterium and control treatments.

3.4. Effects of pathogens on gall formation and nematode reproduction

Table 6 presents field evaluation effects of treatments on total number of galls. Nematode treatment recorded the highest number of galls (33.8 ± 1.74), followed by N + F7 (30.0 ± 4.57) and N + B7 (25.81 ± 3.17) while the control treatment recorded the lowest in gall formation (0.60 ± 0.40). Treatment with fungus or bacteria alone recorded zero number of galls. Friedman's test showed significant differences in the treatment means (S = 36.63, $P < 0.05$). **Table 7** gives the average number of galls per plant and the percentage reduction in galls caused by inoculated pathogenic treatments when compared with the control in field. Treatment with nematode alone gave the highest mean value (6.76 ± 0.35) while the control was the lowest (0.12 ± 0.08) per plant. Treatment with bacterium or fungus recorded no gall at all. Based on the result recorded on the proportional increase in the number of galls using the control as a reference, gall formation in the nematode treatment increased proportionately by 56.33% as the highest recorded, followed by treatment N + F7 with 50.0%. Bambara groundnut roots inoculated with nematode alone had galls slightly higher than that of inoculation of nematode prior to fungi 7 days later compared with uninoculated control. **Figure 2** shows the field evaluation of single and mixed infection effect of treatments on nematode reproduction factor (Rf) Treatment N + F7 had the highest Rf value of 9.30 followed by nematode (8.30), N + B7 (7.40), N + F + B (6.80) and N + F14 (6.50). The least was recorded in N + B14 (6.20) while Zero Rf was recorded in fungus, bacterium and control treatments.

Table 6. Field evaluation of single and mixed infection effects of treatments on number of galls.

Treatments/Blocks	Total B1	Total B2	Total B3	Total B4	Total B5	Grand mean TRT
N	35	36	38	32	28	33.8 ± 1.74^{a}
F	0	0	0	0	0	0.0 ± 0.00^{d}
B	0	0	0	0	0	0.0 ± 0.00^{d}
N + F + B	13	14	12	25	15	15.80 ± 2.35^{c}
N + F7	20	20	30	43	37	30.0 ± 4.57^{ab}
N + B7	22	16	26	33	32	25.8 ± 3.17^{abc}
N + F14	16	18	20	25	26	21.0 ± 1.95^{bc}
N + B14	18	15	17	31	25	21.2 ± 2.97^{bc}
C	0	02	01	0	0	0.60 ± 0.40^{d}
Mean block	13.78 ± 4.00	13.44 ± 3.86	16.00 ± 4.64	21.00 ± 5.53	18.11 ± 4.93	

* Means that do not share same letter are significantly different.
Friedman S (Treatment) =36.63, DF = 8, $P = 0.000$ ($P < 0.05$).

Table 7. Effect of single and mixed infection on average number of gall per plant.

Treatments	Ground mean TRT in all block	Average number galls per plant	% reduction in number of galls against the control
N	33.8 ± 1.74[a]	6.76 ± 0.35	56.33
F	0.0 ± 0.00[d]	0.0 ± 0.00	0.00
B	0.0 ± 0.00[d]	0.0 ± 0.00	0.00
N + F + B	15.80 ± 2.35[c]	3.16 ± 0.47	26.33
N + F7	30.0 ± 4.57[ab]	6.00 ± 0.91	50.00
N + B7	25.8 ± 3.17[abc]	5.16 ± 0.63	43.00
N + F14	21.0 ± 1.95[ab]	4.20 ± 0.39	35.00
N + B14	21.2 ± 2.97[bc]	4.24 ± 0.59	35.33
C	0.60 ± 0.40[d]	0.12 ± 0.08	

* Means that do not share same letter are significantly different.
Friedman S (Treatment) = 36.63, DF = 8, $P = 0.000$ ($P < 0.05$).

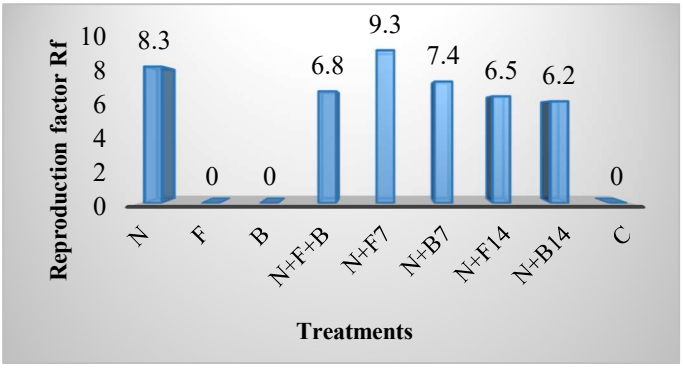

Figure 2. Single and mixed effects of pathogens on nematode reproduction factor (Rf) in Bambara field experiments.

4. Discussion

The observed significant reduction in the number of nodules might have affected pod yield since nodules harbor nitrogen-fixing bacteria in legumes. Root knot nematode infection can cause reduced transport of growth regulators such as cytokinins and gibberellins, thus resulting in reduced growth and yield[24]. The disease complex of the pathogens in Bambara groundnut aggravated symptoms such as wilting, stunted growth and drooping of foliage. Chlorosis was observed in inoculated plants compared to uninoculated control. The effects were observed on leaves of plant inoculated with mixed pathogens. This result agrees with the findings of Iheukwumere et al.[7] who observed mild chlorosis on leaves of plants with combined infection of *M. javanica* and *F. oxysporum*.

The observed chlorosis may be as a result of reduced chlorophyll content and this vividly explains the reduction rate of photosynthesis and yield. This result is similar with the findings of Iheukwumere and Orkpeh[25] who showed that mild chlorosis and wilting occurred in plants infected with both pathogens, and this was attributed to the combined parasitic effects of the fungus and nematode on the plant. The present work found significant difference in the number of plants wilted plants inoculated with fungi alone had the highest wilt percent incidence while plants inoculated with nematod and fungi 7 days germination (N + F7); had difference with that inoculation preceded 14 (N + F14) germination. *Fusarium* has been reported to penetrate xylem elements, aiding in disruption of uptake water minerals by the root system[26] thus, it may account for the wilting and drooping of leaves observed in this study.

The increase number of galls could be as a result of giant cell formation, by the feeding activities of the nematodes which led to excessive root galls. This observation was in conformity with many findings[13,27]. Similarly, the number of galls was apparently higher in plants inoculated with nematode alone probably because, there was no competition with any organism and the nematode had all the necessary space and nutrients to itself for growth and multiplication[7,26]. Formation of galls is the most characteristic symptom of

root knot nematode infection. The nematode secretes growth regulators of the indole group which induces hypertrophy (cell enlargement) and hyperplasia (cell multiplication) at infection or feeding sites[27]. Gall cells serve as nutrient sinks in infected plant and thus cause a shift in the plant metabolism and transport of materials in favour of the roots[9]. This may also be the reason for the observed weight increase. Giant cell formation are triggered off by enzymatic secretions from the root knot nematode in host plants, which induce re-differentiation process that eventually leads to the formation of multinucleated feeding cells called giant cells. It has also been reported that, the presence of nematode infection in plants induces an increase in plant hormones (indole acetic acid, cytokinins, abscisic acid) concentrations which result to accelerated growth around the nematode feeding sites[24,28].

5. Conclusion

Simultaneous infection of Bambara plant with nematode, fungus and bacterium (N + F + B) significantly reduced nodulation. Fungus application caused the highest wilting while nematode application alone induced gall formation, whereas additional inoculation with fungus 7 days after germination (N + F7) gave the highest nematode reproduction factor. The data provided in this work is important in the control of the three pathogens affecting the productivity of Bambara nut. Formulation of a single protectant should be designed to have potent effects on the three pathogens to achieve effective protection and good production of Bambara nut.

Author contribution

Conceptualization, ICC, ACU and NNA; software, OJO and OI; validation, NNA; formal analysis, OJO and NNA; investigation, NNA and ICC; resources, ICC, ACU and NNA; data curation, OJO and OI; writing—original draft, OJO and NNA; writing—review and editing, OT, OI, ICC and ACU; visualization, NNA; supervision, ICC and ACU; project administration, ICC; funding acquisition, NNA. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Hillocks R, Bennett C, Mponda OM. Bambara nut: A review of utilization, market potential and crop improvement. *African Crop Science Journal* 2012; 20(1): 1–16.
2. Olayide OE, Donkoh SA, Ansah IGK, et al. Assessing socioeconomic factors influencing production and commercialization of Bambara groundnut as an indigenous climate resilient crop in Nigeria. In: Leal Filho W (editor). *Handbook of Climate Change Resilience*. Springer Cham; 2018. pp. 1–19. doi: 10.1007/978-3-319-71025-9_158-1.
3. Abejide DR, Falusi OA, Gana AS, et al. Evaluation of seed yield of Nigerian Bambara groundnut [*Vigna subterranea* (L.) Verdc.] landraces under varying water conditions. *Notulae Scientia Biologicae* 2018; 10(2): 233–239. doi: 10.25835/nsb10210238.
4. Tlankka NS, Mbega ER, Ndakidemi PA. Potential of indigenous pesticidal plants in the control of field and post-harvest arthropod pests in Bambara groundnuts (*Vigna subterranea* (L.) Verdc.) in Africa: A Review. *American Journal of Plant Research* 2020; 11: 745–772. doi: 10.4236/ajps.2020.115054.
5. Bamishaiye OM, Adegbola JA, Bamishaiye E1. Bambara groundnut: An under-utilized nut in Africa. *Advances in Agricultural Biotechnology* 2011; 1: 60–72.

6. Agyeman K, Asante BO, Berchie JN, et al. Farmers' perceptions, constraints and preferences for improved Bambara groundnut varieties in Ghana. *Journal of Agriculture and Food Research* 2021; 3: 100097. doi: 10.1016/j.jafr.2020.100097
7. Iheukwumere CC, Dashiell KE, Mutsaers HJM. Infection complex of soybean mosaic potyvirus SMV-10 and *Meloidogyne incognita* and effects on the infectivity and multiplication of both pathogens on soybean. *International Journal of Nematology* 2007; 17(1): 46–54.
8. Al-Hazmi AS, Al-Nadary SN. Interaction between *Meloidogyne incognita* and *Rhizoctonia solani* on green beans. *Saudi Journal of Biological Sciences* 2015; 22(5): 570–574. doi: 10.1016/j.sjbs.2015.04.008
9. Ali WM, Abdel-Mageed MA, Hegazy MGA, et al. Biocontrol agent of root-knot nematode *Meloidogyne javanica* and root-rot fungi, *Fusarium solani* in okra morphological, anatomical characteristics and productivity under greenhouse conditions. *Scientific Reports* 2023; 13: 11103. doi: 10.1038/s41598-023-37837-z
10. Duche TR, Omoigui L, Iheukwumere CC. Variations among *Xanthomonas axonopodis* pv. *vignicola* isolates in Benue State, Nigeria. *International Journal of Innovation and Scientific Research* 2015; 13(1): 271–278.
11. Siddiqui ZA, Fatima M, Alam S. Interactions of *Meloidogyne incognita*, *Xanthomonas campestris*, and *Rhizobium* sp. in the disease complex of chickpea. *Turkish Journal of Agriculture and Forestry* 2013; 37(2): 173–178. doi: 10.3906/tar-1112-48
12. Jagdale GB, Martin K. *Sampling for Plant-Parasitic Nematodes, Identification and Diagnosis*. University of Georgia; 2011.
13. Ashley J, Lindse T. Root knot nematode of soybean (soybean disease formation). Available from: https://content.ces.ncsu.edu/root-knot-nematode-of-soybean (accessed on 8 December 2023).
14. Mohit K, Tripathi UK, Ajay T, Singh PKA. Screening of linseed germplasm for resistance/tolerance against *Farium oxypersom* f sp. Lini (Belley) disease. *Journal of Plant Pathology and Microbiology* 2014; 5(3): 1000235. doi: 10.4172/2157-7471.1000235
15. Alexopoulos CJ, Mims CW, Blackwell MM. *Introductory Mycology*, 4th ed. John Wiley and Sons Inc.; 1996. 880p.
16. Ah-You N, Gagnevin L, Grimont PAD, et al. Polyphasic characterization of *Xanthomonas* pathogenic to members of the Anarcadiaceae and their relatedness to species of *Xanthomonas*. *International Journal of Systemic and Evolutionary Microbiology* 2009; 59(2): 306–318. doi: 10.1099/ijs.0.65453-0
17. Iheukwumere CC, Dashiell KE, Mutsaers HJW. Effects of single and combined infection of soybean mosaic virus and *Meloidogyne incognita* on soybean and replication and pathogenicity of both pathogens. *Nematologia Mediterranea* 2008; 36: 26–30.
18. Bello TT, Fawole B, Abiodun CC. Susceptibility of seven varieties of pepper and tomato to root-knot nematodes (*Meloidogyne* spp.) in Ibadan, Nigeria. *IOSR Journal of Agriculture and Veterinary Science* 2015; 8(1): 79–82. doi: 10.9790/2380-081017982
19. Wonang DL, Akueshi CO. Penetration and development of *Meloidogyne incognita* and *Meloidogyne javanica* in twelve different tomato cultivars in Jos, Nigeria. *African Journal of Natural Sciences* 1998; 2(1): 113–116.
20. Kankam F, Adomako J. Influence of level of root knot nematode (*Meloidgyne* spp.) on tomato (*Solanum lycopersicum* L.). *Asian Journal of Agriculture and Food Sciences* 2014; 2(2).
21. Nene YL, Haware MP, Reddy MV. Chickpea disease resistance—Screening techniques. ICRUISAT; 1981. pp. 1–10.
22. Ismail AE, El-Nagdi WMA, Yasin MY. Histopathology of gall induced chamomile infected with *Meloidogyne incognita* and *Rotylenchus reniformis*. *Pakistan Journal of Nematology* 2004; 22: 143–149.
23. Afolami SO, Atungwu JJ, Odeyemi IS, Orisajo SB. Going beyond gall index in studying and reporting resistance to root-knot nematodes. *Nigeria Journal of Plant Protection* 2004; 21: 25–32.
24. Okechala OB, Wonang DL. The response of eleven sweet potato (*Ipomea batatas* (L.) Lam) cultivars to infection by *Meloidogyne* spp. in Jos, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences* 2015; 10(4): 42–49. doi: 10.9790/3008-10444249
25. Iheukwumere CC, Orkpeh U. Effect of single and mixed infection of *Fusarium oxysporum* (Schlect.) Synder and Hansen and *Meloidogyne javanica* (Treub) Chitwood on growth and yield of soybean (*Glycine max* (L.) Merr.) Var. TGX 1019-2EN. *Nigeran Journal of Botany* 2007; 20(2): 397–406.
26. Kolombia YA, Lava Kumar P, Claudius-Cole AO, et al. First report of *Meloidogyne enterolobii* causing tuber galling damage on white yam (*Dioscorea rotundata*) in Nigeria. *Plant Disease* 2016; 100(10): 2173. doi: 10.1094/PDIS-03-16-0348-PDN
27. Zongo E, Néya BJ, Traoré VSE, et al. Impact of *Cowpea mottle virus* on the growth and yield of Bambara Groundnut (*Vigna subterranean* (L.) Verdc.). *American Journal of Plant Science* 2018; 9: 2053–2062. doi: 10.423/ajps.2018.910149
28. Koutsovoulos GD, Poullet M, Elashry A, et al. Genome assembly and annotation of *Meloidogyne enterolobii*, an emerging parthenogenetic root-knot nematode. *Scientific Data* 2020; 7(1): 324. doi: 10.1038/s41597-020-00666-0