

EFFECTS OF SINGLE AND MIXED INFECTION OF MELOIDOGYNE JAVANICA (TREUB) CHITWOOD AND FUSARIUM OXYSPORUM (SCHLECHT) SYNDER AND HANSEN ON GROWTH AND YIELD PARAMETERS OF BAMBARA GROUNDNUT (VIGNA SUBTERRENEA (L.) VERDC.

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ABSTRACT

The aim of the investigation was to determine the pathogenicity of *Nematode-N* (*Meloidogyne javanica*) and *Fungus-F* (*Fusarium oxysporum*) in Bambara groundnut (*Vigna subterreneae* (L.) Verdc.) under field condition using growth and yield parameters. Fungus was sourced from infested plants while nematode was sourced from infested soils using standard procedures. The Randomized Complete Block Design (RCBD) consisting 5 replicates, 6 treatments, 5 blocks and a total of 150 experimental units. Inoculation of pathogens into Bambara plants was done followed by plant characterization at flowering and maturity stage. Results showed that simultaneous infection by N+F pathogens caused the highest percentage loss in flowering (64.76%), number of pods (75.57%), and weight parameters. This was followed by other mixed treatments in the descending order N+F7, N+F14. The damages caused by mixed interactions of pathogens were higher than the single effects, the least being the fungus. Therefore, inoculation of nematode, fungus simultaneously (N+F) caused significant damages on flowering and yield components of Bambara nut field trials. This finding is crucial in the disease management of Bambara groundnut to enhance its yield and overall productivity to achieve food security.

INTRODUCTION

Vigna subterreneae var. *spontanea* (wild varieties) and *Vigna subterreneae* var. *subterreneae* (cultivated varieties) are the two botanical varieties of Bambara groundnut (Agyeman *et al.*, 2021). Most of the world's Bambara groundnut is grown in West Africa and the crop is most prominent in the traditions of rural communities (Hillocks *et al.*, 2012). Bambara groundnut is important for small holders and their households because the beans are sources of vital nutrients including high protein content (Hillocks *et al.*, 2012; Yao, *et al.*, 2015; Atoyebi *et al.*, 2018;

Unigwe *et al.*, 2018; Feldman *et al.*, 2019). Compared to other legumes, Bambara nut is deficient in sulphur-containing amino acids while some genotypes contain higher amount of methionine and lysine (Unigwe *et al.*, 2018). Tan *et al.* (2020) described Bambara as an underutilized leguminous crop for global food security and nutrition. Bambara groundnut seeds contain 63% carbohydrates, 19% protein and 6.5% oil (Hillocks *et al.*, 2012; Tan *et al.*, 2020). The gross energy value of Bambara nut seeds is greater than that of other pulses viz; cowpea (*Vigna unguiculata*), lentil (*Lens esculenta*) and pigeon pea (*Cajanus cajan*), (FAO, 2015). It is a good source of fibre, Calcium (Ca), Iron (Fe) and Potassium (K) (Hillocks *et al.*, 2012; Tan *et al.*, 2020).

The production of Bambara groundnut is challenged by biotic stresses. Noteworthy fungal diseases are Cercospora leaf spot (CLS) caused by *Cercospora* spp., Powdery mildew (*Erysiphe polygoni*), Fusarium wilt (*Fusarium oxysporum*), Rust (*Puccinia* spp) and Leaf blight (*Colletotrichum* spp). Bacterial blight (*Xanthomonas* spp) and Bacterial wilt (*Ralstonia solanacearum*) are among the bacterial pathogens that infect Bambara groundnut (Majola *et al.*, 2021; Waleed *et al.*, 2023). Among the nematode diseases, root knot nematode, *Meloidogyne javanica* (Treub) Chitwood, is the most dominant species that causes severe losses to Bambara yield (Meena *et al.*, 2016; Majola *et al.*, 2021). Other species of *Meloidogyne* including *M. enterolobii* and *M. incognita* have been reported to parasitize legumes (Chitambo *et al.*, 2016; Kassam *et al.*, 2022). Root knot nematodes (RKNs) are one of the most important nematode pests of crop plants and have diverse host range (Sayan *et al.*, 2008; Waleed *et al.*, 2023). They constitute major constraints to the cultivation and production of crops worldwide including Nigeria (Olayide *et al.*, 2018; Patil *et al.*, 2020).

Plant parasitic nematodes are known to aggravate the final yield losses as they form disease complexes through their interactions with fungi, bacteria and viruses. In the rhizosphere, root knot nematodes interact with a wide range of microbiota/plant pathogens viz fungi, bacteria, viruses (Al-Hazmi and Al-Nadary, 2015). The combined effects of wilt causing fungi, bacteria and root knot nematodes cause serious damage to crops. With the worldwide distribution, extensive host range and interaction with fungi, bacteria and viruses in disease complexes, root knot nematodes rank first among the ten damaging genera of parasitic nematodes affecting world food supply (Khpalwak, 2012). Information is lacking on the single and combined interaction effects of three common pathogens on the production of Bambara crop. The aim of the present study was to determine the nature of pathogenicity of nematode (*Meloidogyne javanica*), fungus (*Fusarium oxysporum*) and bacterium (*Xanthomonas axonopodis*) as they affect the flowering and yield of Bambara groundnut (*Vigna subterrene* (L.) Verdc.) under field condition.

MATERIALS AND METHODS

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" North and 8° 32' 10" East). 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 (NAF, 2020). The field used for this investigation had previously been cropped to tomato and egg plants with high *Meloidogyne javanica* infestation but had no incidence of *Fusarium oxysporum* infection.

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.

Sterilization of seeds, glass ware and soil

Seeds were surface sterilized using 1.08% Sodium hypochlorite solution (Ganpati and Judy, 2014). Other materials were either air dried as in plastics or oven dried as in glass wares or autoclaved as in media (Khpalwak, 2012). All laboratory activities were carried out under aseptic condition (Khpalwak, 2012)

Sources of test pathogens

Fusarium oxysporum was isolated from stem and root of diseased okra (*Abelmoschus esculentus* L.) using the method of (Iheukwumere *et al.* (2009) and sub-cultured on Potato Dextrose Agar (PDA) plates (Mohit *et al.*, 2014) followed by morphological identification on the stereobinocular microscope and the use of identification guide (Alexopoulos *et al.*, 2002). Spores were separated from the mycelium. *Meloidogyne javanica* was collected from galled roots of infected tomato plants (*Solanum lycopersicum* L.) in the field using standard methods (Ganpati and Judy, 2014). The infected roots were examined for the presence of galls containing adult female and juvenile (Iheukwumere *et al.*, 2008; Bello *et al.*, 2015) using perineal pattern morphology as compared with standard pictorial guide (Wonang and Akueshi, 1999).

Field experimental design and seed sowing

The field was cleared, ploughed and planting beds prepared. The trial was arranged in a Randomized Complete Block Design (RCBD) with five blocks each measuring 13m x 2m spaced 1m from each other. Each block consisted of nine subplots which measured 2m x 1m and each plot consists of five rows of plants spaced 40cm apart. The experiment was a 5*6*5 factorial design (5 blocks, 6 treatments, 5 replicates per treatments) resulting in 150 experimental units. Figure 2 shows the field experimental layout. In each experimental unit, three seed of Bambara groundnut were sown to a depth of 3-5cm and thinned to one plant per planting hole after germination at day 7. In doing this, care was taken to ensure that the plants were of uniform growth and vigour (Bello *et al.*, 2014).

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 as previously described in the pot experiment. The fungus was inoculated as described in the pot culture experiment. The nematicide Carbofuran is a granular formulation that was incorporated into the top 10 to 15cm of soil at the rate of 3kg per hectare prior to sowing. This nematicide is highly effective in the control of root knot nematodes, *Meloidogyne* spp (Kankam and Adomako, 2014). The experiment was terminated 120 days after inoculation when the plants had fully matured.

Treatment combination and description

T1: Bambara seedlings exposed to natural infection by the nematode in the soil at planting (N)

T2: Bambara seedlings inoculated with 10 cfu/ml of fungus alone (F) after sowing in Carbofuran (Furadan 10g) treated soil (F)

T3: Seedlings exposed to natural infection by nematode in the soil followed by 10 cfu/ml of fungus suspension simultaneously (N+F)

T4: Seedlings exposed to natural infection by nematode in the soil followed by inoculation with 10 cfu/ml of fungus 7 days after germination (N+F7)

T5: Seedlings exposed to natural infection by the nematode in the soil followed by inoculation with 10 cfu/ml of fungus 14 days after germination (N+F14)

T6: Seedlings with no nematode, fungus or bacterium in plots treated with Carbofuran (Furadan 10G) which served as control (C).

Growth and yield characterization

Yield parameters were determined through standard measurement of length using the meter rule (cm) or weight using digital weighing balance (g) or by direct counting (Iheukwumere and Orkpeh, 2007). The following parameters were determined: shoot fresh and dry weight (g), root fresh and dry weight (g), number of flowers and number of pods.

Data analysis

Minitab 17.0 software was used to analyse the data collected. Descriptive statistical tools were applied while Two Way ANOVA tool was employed in the determination of treatments and block effects across the various parameters investigated in the field. One Way ANOVA tool was used in the determination of treatment effects in the pot experiments. Means separation was done using Fisher's LSD methods at 95% level of confidence. Friedman and Kruskal Wallis tests were applied as non-parametric tool

RESULTS AND DISCUSSION

Table 1 presents field evaluation effects of single and mixed infection of root knot nematode (*M. javanica*), fungus (*F. oxysporum*) on total number of flowers as a phenological parameter. The control treatment recorded the highest number of flowers counted (94.2 ± 7.62) followed by nematode treatment (54.8 ± 6.54) while the combined treatment N+F had the smallest value (33.2 ± 2.82). Table 2 gives the average number of flowers per plant and the percentage reduction in flower production caused by inoculated pathogens using the control as a reference. The control treatment recorded the highest with mean of 18.84 ± 1.52 while treatment N+F was the least with an average mean of 6.64 ± 0.56 per plant. As a result, treatment N+F caused the highest percentage reduction in flower (64.76%), followed by N+F7 (52.55%), and bacterium (39.2%). Flowers are the organs of reproduction in angiosperms that accounts for plant yields through fertilization of ovules and development of ovaries to form seeds and fruits respectively (Mkandawire, 2017). The result obtained aligned with the findings of Waleed *et al.* (2023) who found that single and interaction effects of pathogens had drastic effects on flowering of legumes, and that pod size can be reduced significantly if infestation occurs before flowering. The observed reduction in flowering might be as a result of impaired supply of Nitrogen, Potassium and Phosphorus in plants as a result of pathogenic activities which resulted to delayed flowering and flowers abortion (Onyeke and Akueshi, 2011).

Table 1: Field Evaluation of Single and Mixed Infection Effects of Treatments on Number of Flowers

Treatments/Block	Total B1	Total B2	Total B3	Total B4	Total B5	Grand Mean TR
N	80	55	45	46	48	54.8 ± 6.54^b
F	76	57	48	51	34	53.2 ± 6.84^b
N+F	37	36	35	36	22	33.2 ± 2.82^c
N+F7	65	41	42	40	35	44.6 ± 5.24^b
N+F14	71	53	46	44	38	50.4 ± 5.68^b
C	124	92	87	82	86	94.2 ± 7.62^a

Mean Block	75.11±7.51	54.33±5.41	50.11±4.91	48.56±4.48	41.33±6.03
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* Means that do not share same letter are significantly different
 F (Treatment) = 55.57, P= 0.000 (P<0.05)
 F (Block) =55.4, P=0.000 (P<0.05)

Table 2: Effect of Single and Mixed Infection on Average Number of Flower per Plant

TREATMENTS	Ground Mean TRT in all Block	Average number of flower per plant	% reduction in number of flowers against control
N	54.8±6.54 ^b	10.96±1.31	41.83
F	53.2±6.84 ^b	10.64±1.37	43.52
N+F	33.2±2.82 ^c	6.64±0.56	64.76
N+F7	44.6±5.24 ^{bc}	8.92±1.05	52.55
N+F14	50.4±5.68 ^{bc}	10.08±1.14	46.50
C	94.2±7.62 ^a	18.84±1.52	Reference point

* Means that do not share same letter are significantly different

F (Treatment) =55.57, P= 0.000 (P<0.05)

F (Block) = 55.4, P=0.000 (P<0.05)

Table 3 presents field evaluation effects of single and mixed infection of root knot nematode (*M. javanica*), fungus (*F. oxysporum*) on total number of pods as a yield parameter. The control treatment had the highest mean number of pods (74.8±3.34), and nematode (41.8±5.56) as single interaction. Combined or simultaneous treatment N+F recorded the least number of pods (18.2±1.32). The result showed significant difference in treatment (F=38.4, P<0.05) and block (F=9.98, P<0.05) effects. Table 4 gives the average number of pods per plant and the percentage reduction in pod production caused by inoculated pathogens using the control as a reference. The control treatment recorded the highest with mean of 14.96±0.67 while treatment N+F was the least with an average mean of 3.64±0.26 per plant. Consequently, treatment N+F caused the highest percentage and significant reduction in pod production (75.57%) followed by N+F7 (54.55%).

These pathogens might have certainly altered the physiological processes in the plant thereby negatively impacting on its yield. This observation tallies with the findings of Iheukwumere (2007) and Waleed *et al.* (2023). The reduction in yield, presence of chlorotic regions or patches and stunting of the inoculated plants may be attributed to lack of physiological stability, as a result of reduced translocation, impaired nutrient absorption and abnormal production of growth regulators (Okechalu and Wonang, 2015; Meena *et al.*, 2016; Collett *et al.*, 2021). The parasitic effect of the infected roots by soil borne pathogens causes water stress and induces physiological responses that lead to the partitioning of carbohydrates in plant organs, reduction of photosynthetic processes and decreased efficiency of carbon-fixation (Meena *et al.*, 2016; Zongo *et al.*, 2018; El-Sayed, 2022).

Table 3: Field Evaluation of Single and Mixed Infection Effects of Treatments on Number of Pods

Treatments/Block	Total B1	Total B2	Total B3	Total B4	Total B5	Grand Mean TRT
N	41	40	43	44	25	38.60±3.47
F	30	42	51	57	29	41.8±5.56 ^b
N+F	19	19	17	22	14	18.2±1.32 ^c
N+F7	40	30	38	40	22	34.0±3.52 ^b

N+F14	36	38	40	42	36	38.4±1.17 ^b
C	87	68	70	76	73	74.8±3.34 ^a
Mean Block	40.78±6.21	38.78±4.38	43.33±4.78	47.22±4.87	32.11±5.53	

* Means that do not share same letter are significantly different

F (Treatment) = 38.4, P= 0.000 (P<0.05)

F (Block) =9.98, P=0.000 (P<0.05)

Table 4: Effect of Single and Mixed Infection on Average Number of Pods per Plant

TREATMENTS	Ground Mean TRT in all Block	Average number Of leaves per plant	% Reduction in number of leaves against Control
N	38.60±3.47 ^b	7.72±0.69	48.40
F	41.8±5.56 ^b	8.36±1.11	44.12
N+F	18.2±1.32 ^c	3.64±0.26	75.57
N+F7	34.0±3.52 ^b	6.8±0.70	54.55
N+F14	38.4±1.17 ^b	7.68±0.23	48.66
C	74.8±3.34 ^a	14.96±0.67	

* Means that do not share same letter are significantly different

F (Treatment) =38.4, P= 0.000 (P<0.05)

F (Block) = 9.98, P=0.000 (P<0.05)

Table 5 presents field evaluation effects of single and mixed infection of root knot nematode (*M. javanica*), fungus (*F. oxysporum*) and on total pod fresh weight (g) as a yield parameter. The control treatment had the highest mean fresh pod weight (317.7±18.1g), followed by fungus (181.0±18.9g). Combined treatment N+F recorded the least number of pods (95.2±6.12g). The result showed significant difference in treatment (F=36.0, P<0.05) and block (F= 7.17, P<0.05) effects. Table 6 gives the average fresh pod weight per plant and the percentage reduction in fresh pod weight caused by inoculated pathogens using the control as a reference. The control treatment recorded the highest with mean of 63.54±3.62 while treatment N+F was the least with an average mean of 19.04±1.22 per plant. Consequently, treatment N+F caused the highest percentage and significant reduction in fresh pod weight (70.0 %) followed by N+F7 (49.92%). The present observation is in consonance with the findings of Iheukwumere *et al.* (2007) who showed that the parasitry effects of both pathogens on the roots of the plant impacted more negatively on the plant than infection with each pathogen alone. The present finding also aligns with the work Kanjo and Iheukwumere (2021) who found a significant reduction in growth components of susceptible and to an extent, resistant tomato plants under the influence of single and mixed infection of *F. oxysporum* and *M. javanica*.

Table 5: Field Evaluation of Single and Mixed Infection Effects of Treatments on Pod Fresh Weight (g)

Treatments/Block	Total B1	Total B2	Total B3	Total B4	Total B5	Grand Mean TRT
N	198.00	204.30	167.60	194.10	127.50	177.7±13.4
F	139.20	203.50	178.40	240.20	143.80	181.0±18.9
N+F	103.20	98.50	88.50	110.50	75.30	95.2±6.12 ^c
N+F7	174.00	170.60	161.80	177.60	111.40	159.1±12.2
N+F14	168.70	165.20	171.60	185.60	173.40	172.9±3.47
C	381.70	278.00	289.10	322.90	316.60	317.7±18.1
Mean Block	188.7±25.9	187.3±15.7	174.8±52.0	206.5±19.1	154.7±22.4	

* Means that do not share same letter are significantly different

F (Treatment) = 36.00, P= 0.000 (P<0.05)

F (Block) =7.17, P=0.000 (P<0.05)

Table 6: Effect of Single and Mixed Infection on Average Number of Pod Fresh Weight per Plant

TREATMENTS	Ground Mean TRT in all Block	Average pod weight per plant	% Reduction in pod weight against Control
N	177.7±13.4 ^b	35.5±2.68	44.07
F	181.0±18.9 ^b	36.2±3.78	43.03
N+F	95.20±6.12 ^c	19.04±1.22	70.03
N+F7	159.1±12.2 ^b	31.82±2.44	49.92
N+F14	172.9±3.47 ^b	34.58±0.69	45.58
C	317.7±18.1 ^a	63.54±3.62	Reference

F (Treatment) =36.00, P= 0.000 (P<0.05)

F (Block) = 7.17, P=0.000 (P<0.05)

* Means that do not share same letter are significantly different

Table 7 presents field evaluation effects of single and mixed infection of root knot nematode (*M. javanica*), fungus (*F. oxysporum*) on total pod dry weight (g) as a yield parameter. The control treatment had the highest mean fresh pod weight (89.16±4.16g). Combined treatment N+F recorded the lowest value (23.68±1.34g). The result showed significant difference in treatment (F=31.97, P<0.05) and block (F= 7.88, P<0.05) effects. Table 8 gives the average dry pod weight per plant and the percentage reduction in dry pod weight caused by inoculated pathogens using the control as a reference. The control treatment recorded the highest with mean of 17.88±0.84 while treatment N+F was the least with an average mean of 4.74±0.27 per plant. Consequently, treatment N+F caused the highest percentage and significant reduction in fresh pod weight (73.49 %) followed by N+F7 (51.45%), and. Figure 1 presents field evaluation effects of single and mixed infection of root knot nematode (*M. javanica*), fungus (*F. oxysporum*) on % reduction on number of flowers, number of pods and pod fresh weight at different treatments. Treatment N+F recorded the highest reduction in three yield parameters. This was followed by mixed treatments in the descending order N+F7, N+F14. These effects caused by mixed interactions were higher than the single treatments, the least being the bacterium inoculum. The manifestation by mineral deficiency symptoms by bambara groundnut infected with *Meloidogyne javanica* and *Fusarium oxysporum* can therefore be attributed to impaired translocation of these elements by the activities of root knot nematodes which feed and block vascular tissues of their host (Waleed *et al.*, 2023).

Table 7: Field Evaluation of Single and Mixed Infection Effects of Treatments on Pod Dry Weight (g)

Treatments/Block	Total B1	Total B2	Total B3	Total B4	Total B5	Grand Mean TRT
N	50.50	49.20	52.60	53.70	30.80	47.36±4.21 ⁱ
F	36.80	51.20	61.80	68.80	38.60	51.44±6.28 ^j
N+F	24.10	24.70	22.80	27.50	19.30	23.68±1.34 ^k
N+F7	48.40	37.30	47.10	56.60	27.50	43.38±5.0 ^b
N+F14	44.00	46.70	49.10	51.30	49.20	48.06±1.25 ^l
C	104.30	81.90	81.30	90.80	88.70	89.4±16 ^a
Mean Block	50.0±7.33 ⁱ	52.54±7.31 ⁱ	52.94±5.22 ⁱ	58.19±5.61 ⁱ	41.13±6.63	

* Means that do not share same letter are significantly different
 F (Treatment) = 31.97, P= 0.000 (P<0.05)
 F (Block) =7.88, P=0.000 (P<0.05)

Table 8: Effect of Single and Mixed Infection on Average Pod Dry Weight and Percentage Reduction in Dry Weight per Plant

TREATMENTS	Ground Mean TRT in all Block	Average number Of leaves per plant	% Reduction in number of leaves against Control
N	47.36±4.21 ^b	9.47±0.84	47.04
F	51.44±6.28 ^b	10.29±1.26	42.45
N+F	23.68±1.34 ^c	4.74±0.27	73.49
N+F7	43.38±5.01 ^b	8.68±1.00	51.45
N+F14	48.06±1.25 ^b	9.61±0.25	46.25
C	89.4±4.16 ^a	17.88±0.84	-

* Means that do not share same letter are significantly different
 F (Treatment) =31.97, P= 0.000 (P<0.05)
 F (Block) = 7.88, P=0.000 (P<0.05)

CONCLUSION

The investigation revealed that damages caused by mixed interactions of pathogens were higher than the single effects, nematode (N) and Fungus n(F). Therefore, inoculation of nematode, fu simultaneously (N+F) caused significant damages on growth and yield components of Bambara nut field trials. This report is crucial in the disease management of Bambara groundnut to enhance its yield and overall productivity to achieve food security.

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