INDIVIDUAL AND INTERACTIVE UTILITY OF BIOLOGICAL AND PHYSICAL INVIGORATION FOR VARIOUS CARROTS SEEDS ORDERS AND STUDY THEIR FIELD PERFORMANCE

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ABSTRACT

The purpose of this research is assessing the significance of biological and physical seeds invigoration of different aged carrot seeds orders and study their field performance. An implementation of field experiment at station (A) College of Agricultural Engineering Sciences - University of Baghdad during fall season 2021-2022. The experimental design arranged using split plot within RCBD. As carrot seeds order considered main plots (primary P, secondary S, tertiary T) and seeds invigoration treatments sub plots (untreated seeds C0, biological B, Physical G, B+G) with three replicates (3X4X3). The interaction treatment PBG (biological+physical treated primary seeds order) reveals significance in majority of research traits such as field emergence percent, carrot total carotenoids, scavenging percent, total yield (97.67%,12.66mg.100g, 88%, 126.1 ton.ha^{-1}) respectively in compare to untreated tertiary seeds order (TC0).

Keywords: mycorrhiza; trichoderma; gamma ray; sustainability; endo-β-mannanase

الفائدة الفردية والتفاعلية للتنشيط الاحيائي والفيزيائي لرتب مختلفة من بذور الجزر ودراسة ادائها الحقلي

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المستخلص

هدف البحث الى تقييم مدى تأثير معاملات تنشيط البذور الاحيائية والفيزيائية في رتب مختلفة من بذور جزر قديمة ودراسة ادائها الحقلي ،نفذت التجربة الحقلية في محطة A في كلية علوم الهندسة الزراعية/جامعة بغداد للموسم الخريفي 2022- 2021، تم ترتيب التصميم التجريبي على وفق القطع المنشقة باستعمال تصميم القطاعات الكاملة المعشاة، تم توزيع رتب البذور على القطع الرئيسة (اولية P، ثانوية S، ثالثية T)، وتوزيع معاملات تنشيط البذور على القطع الثانوية (بذور غير معاملة C0، تنشيط احيائي B، تنشيط فيزيائي G، كليهما BG)، أوضحت النتائج التفوق المعنوي لمعاملة التداخل لبذور الرتبة الاولى المعاملة فيزيائيا وكيميائيا (PBG) لمعظم صفات الدراسة مثل النسبة المئوية للبزوغ الحقلي والكاروتينات الكلية في الجزر والنسبة المئوية للكسح والحاصل الكلي (97.67%، 12.66 ملغم.100 غم$^{-1}$، 88%، 126.1 طن. هـ$^{-1}$) على التتابع، مقارنة بمعاملة بذور الرتبة الثالثية غير المعاملة TC0.

الكلمات المفتاحية: مايكورايزا، ترايكوديرما, اشعة كاما, استدامة, اندو بيتا مانانيز.

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INTRODUCTION

Carrot plant *Daucus carota* was utilized as food after initially being employed for medicinal purposes for decades (44). Its unique combination from vitamins, antioxidants, minerals, phytochemicals and pigments has given the mentioned therapeutic value. However; carotenoids are the most prominent feature that highlighted its significance in diseases preventive nutrition (24, 36, 40, 42). As a result; many studies addressed its reproductive biology (2, 4, 23, 33), in particular; seed quality enhancement (27, 32, 34 , 48). A remarkable phenomenon that could be observed in mature carrot seeds after the dispersal from the maternal plant; which demonstrated by possessing immature embryos (15, 38, 29). Unlike most of plants seeds; carrot rudiment embryos complete their development after imbibition (26). The sharpness of rudimentary embryo is directly proportional to umbel order that's seeds came from. In fact; it's slight in primary umbels seeds. Consequently; the more umbels order increase; the more the sharpness rises (41). Many scholars used breaking down enzymes activity as an indicators for completing embryo maturity, development and uniformizing emergence (13, 47). Consequently; using pre-sowing treatments that specifically targeting the embryo development and breaking down enzymes in seeds is the whole point. endo-β-mannanase is the main enzyme that fundamentally responsible of galactomannans degredation (11). In fact; galactomannans functions as a barricade that limits embryo growth in many plant species (22). Hence the mentioned enzyme activity regarded a solid proof of embryo growth. In recent years; it becomes fairly prevalent to apply multipurpose and clean fertilizers (3, 6). Fungi biofertilizers were the most recommended since they function via a range of processes. For example; seed invigoration, pest's prevention, greater nutrients intake, and soil repair (5). Monalisa et al (35) observed that bio-priming common bean seeds with *Trichoderma viride, Trichoderma harzianum* that resulted in improvement in germinaton speed, shoot and root length, and seedling dry matter. Another results obtained by (8) exhibited greater emergence of carrot seeds when treated with beneficial microorganisms. Regarding seed technology; physical invigoration of seeds can now have several levels of advantages such as structure affecting, gene expression changing, storability extending, and metabolites accumulating (7, 18, 43, 46, 50). Gamma rays have been demonstrating magnificent results at low doses relating to raising seed germination and uniformity in various plant species (28, 49). Bovi et al (9) observed noticeable increase in carrot weight when exposing seeds to gamma ray at doses 2.5, 10 Gy. h^{-1}. A further investigation done by Holonec et al (20) on sessile oak seeds by delivering gamma rays at 2 Gy. h^{-1}; showing enhancement in germination speed and percent. As a result; this experiment was attempting to fix and regulate emergence of different carrot seeds orders by biological and physical invigoration and how that reflect on future plant field performance and quality.

MATERIALS AND METHODS

Seeds order source

Carrot seeds Var. Nantes was obtained from scientific research station (A) at College of Agricultural Engineering Sciences/ University of Baghdad, in which seeds harvested separately according to umbels order. The seeds age were thirty months (30 m0nths). Thus, they are essentially considered old. The symbolization of seeds orders was as follows: First order (P), second order (S), third order (T).

Pre-sowing seeds invigoration treatments preparation

Biological seeds invigoration: this practice implemented at plant protection laboratory/ Ministry of Science and Technology. Carrot seeds were soaked to fungal inoculants solution (*Tricoderma asperellum +Glomus intraradices*+ gum Arabic as adhesive agent + distilled water) for half an hour. The symbolization of biological treating was (B). Physical seeds invigoration: seeds exposure to gamma radiation carried out at radiation laboratory/ physics dept./College of Science/ University of Baghdad. Every single seed received 10 Gy. 1 h^{-1}. The symbolization of gamma treating was (G). As for BG treatment; gamma ray exposure is done first after that the

irradiated seeds soaked at the mentioned inoculation solution.

Determination of endo-β-mannanase enzyme activity (µmol.reducing sugar equivalents $min^{-1}.g^{-1}$f.w.):

this measurement carried out at enzymes laboratory/ Institute of Genetic Engineering and Biotechnology/ University of Baghdad. The mentioned enzyme directly determined on imbibed carrot seeds after the completion of seeds invigoration treatments, i.e.; before field cultivation. It determined by spectrophotometer at wavelength 398 nm according to (39).

Field experiment

Field experiment was conducted at station (A) during agricultural fall season 2021- 2022. The treated carrot seeds sowed according to the mentioned treatments in 22/9/2021. The cultivation was on terraces with three lines. Seeds were spaced 5cm apart, and lines were spaced 25 cm apart. Plant density was 800000 $plant.ha^{-1}$. The field was under drip irrigation system. The experiment arranged as a split plot design with RCBD in three replication (3X4X3), in which seeds order distributed in main-plots and seed invigoration treatments distributed on sub-plots. The means were compared according to LSD (5%). Carrots harvested from all plots in 5/1/2022.

The study traits included determination of field emergence (%): it counted in the field during 30 days from sowing date for 100 cultivated seeds. plant length (cm), leaves number.$plant^{-1}$, canopy dry weight (g.), chlorophyll concentration in carrot leaves (mg.100g fw^{-1}) according to (16), total carotenoids in carrots (mg.$100g^{-1}$) based on (17) method, DPPH scavenging assay (%) according to (25), carrot weight (g.) total yield (ton.ha^{-1}). Determination of DPPH scavenging assay (%) carried out at enzymes laboratory/ Institute of Genetic Engineering and Biotechnology/ University of Baghdad, as for chlorophyll conc. Carrot leaves, and total carotenoids in carrots; they determined at plant nutrition laboratory/ College of Agricultural Engineering Sciences/ University of Baghdad.

RESULTS AND DISCUSSION

endo-β-mannanase enzyme activity (µmol.reducing sugar equivalents $min^{-1}.g^{-1}$f.w.): It's noticeable from figure 1 that first and second order carrot seeds had the higher activity of endo-β-mannanase enzyme in compare to third order. As for invigoration treatments; gamma ray (G) had higher enzyme activity than the biological treated seeds (B) for all seeds orders. However; biological seeds invigoration+ gamma ray (GB) had the most elevated enzyme activity in compare with gamma ray receiving only (G) or biological invigoration (B) or control (CO). The best enzyme activity obtained from PBG treatment followed by SBG treatment.

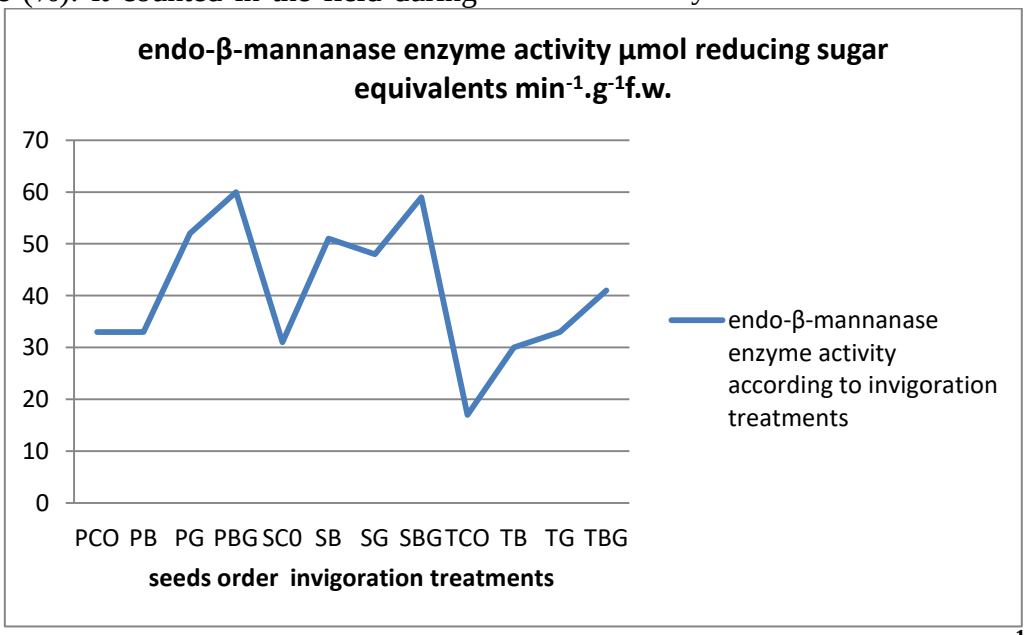

Figure 1. Endo-β-mannanase enzyme activity µmol.reducing sugar equivalents $min^{-1}.g^{-1}$f.w. as affected by carrot seeds invigoration treatments after 24h imbibition

Field emergence (%) and canopy treats:

Results in table (1) reveals significant superiority of primary umbels seeds (P) by producing the highest field emergence percent, plant length, Leaves number, canopy dry weight, and leaves chlorophyll conc. (91.32%, 81.17 cm, 8.92 leaf. plant^{-1}, 3.67 g, 369.6 mg.100g fw^{-1}) respectively, in compare with the lowest that found in tertiary umbels seeds (T) (77.58%, 66.33 cm, 6.42 leaf. plant^{-1}, 1.78 g, 290.4 mg.100g fw^{-1}) respectively.

The impact of seeds invigoration treatments is significantly clear in favor of biological seeds invigoration+ gamma ray (BG) which produced the highest results in compare with untreated seeds (CO) (table 1). Concerning the interaction treatments; the best significant results were obviously found in primary umbels seeds + biological seeds invigoration with gamma ray (PBG) (97.67%, 10.66 leaf. plant^{-1}, 5.33 g, 394.7mg.100g fw^{-1}) respectively. Conversely; the lowest that exist in untreated third order seeds (TC0) (72.33%, 5.67 leaf. plant^{-1}, 1.33 g, 284mg.100g fw^{-1}) (table 1).

Table 1. invigorating different carrot seeds orders by biological and physical invigoration for various carrots seeds orders on field emergence (%) canopy traits

Traits treatments	Field emergence (%)	Plant length (cm)	Leaves number.plant^{-1}	Canopy dry weight (g.)	Chlorophyll conc. (mg.100g fw^{-1})
Seeds order					
P	**91.32**	**81.17**	**8.92**	**3.67**	**369.6**
S	**89.83**	**74.08**	**8.58**	**3.28**	**317.2**
T	**77.58**	**66.33**	**6.42**	**1.78**	**290.4**
LSD 0.05	**1.489**	**0.500**	**0.38**	**0.719**	**4.876**
Seeds invigoration treatments					
C0	**81.11**	**69.78**	**7.33**	**2.16**	**308.6**
B	**88.00**	**75.11**	**8.11**	**2.99**	**330.0**
G	**85.33**	**71.22**	**7.11**	**2.44**	**316.3**
BG	**90.56**	**79.33**	**9.33**	**4.06**	**348.0**
LSD 0.05	**1.777**	**1.713**	**0.67**	**0.771**	**8.058**
Seeds order x Seeds invigoration treatments					
PC0	**86.00**	**77.67**	**8.33**	**2.67**	**344.7**
PB	**91.33**	**82.67**	**9.00**	**4.00**	**375.0**
PG	**90.33**	**77.00**	**7.67**	**2.68**	**364.0**
PBG	**97.67**	**87.33**	**10.66**	**5.33**	**394.7**
SC0	**85.00**	**71.00**	**8**	**2.47**	**297.0**
SB	**91.34**	**74.00**	**8.33**	**3.00**	**320.0**
SG	**89.00**	**72.00**	**7.67**	**2.68**	**302.0**
SBG	**94.00**	**79.33**	**10.32**	**4.68**	**350.0**
TC0	**72.33**	**60.67**	**5.67**	**1.33**	**284.0**
TB	**80.33**	**68.68**	**7.00**	**1.97**	**295.0**
TG	**76.67**	**64.66**	**6.00**	**1.67**	**283.0**
TBG	**81.33**	**71.32**	**7.00**	**2.17**	**299.4**
LSD 0.05	**2.580**	**N.S.**	**1.17**	**1.262**	**9.594**

Quality and yield traits:

table 2 reveals significant impact of primary umbels seeds (P) to have the potential to produce the largest amount of carotenoids per 100 from carrots (11.71 mg), and highest scavenging percent (78.75%), even more; the mentioned treatment shows significant superiority in individual carrots (129.1g) and total yield (103.3 ton.ha^{-1}) in compare with tertiary umbels seeds (T) which produced the lowest. The influence of seeds invigoration treatments is notably favorable to biological seeds invigoration+ gamma ray (BG) which produced the highest results in comparing with untreated seeds (CO) (table 2). With regard to interaction treatments; primary umbels seeds + biological seeds invigoration with gamma ray (PBG) produced the highest quality and yield

traits (12.66mg.100g, 88%, 157.7g, 126.1 ton.ha^{-1}) respectively. On the other hand; untreated third order seeds (TC0) have the lowest (9.33mg.100g, 59%, 75.7g, 60.53 ton.ha^{-1}) (table 2).

Table 2. invigorating different carrot seeds orders by biological and physical treatments for various carrots seeds orders on quality and yield traits

Traits treatments	Total carotenoids (mg.100g^{-1})	DPPH scavenging (%)	Carrot weight (g.)	Total yield (ton.ha^{-1})
Seeds order				
P	**11.71**	**78.75**	**129.1**	**103.3**
S	**11.58**	**74.92**	**123.5**	**98.80**
T	**9.50**	**64.25**	**79.40**	**63.53**
LSD 0.05	**0.428**	**3.199**	**4.03**	**3.227**
Seeds invigoration treatments				
C0	**10.50**	**66.33**	**92.70**	**74.13**
B	**11.22**	**74.56**	**115.2**	**92.18**
G	**10.22**	**70.44**	**103.0**	**82.40**
BG	**11.78**	**79.22**	**131.8**	**105.4**
LSD 0.05	**0.443**	**3.302**	**6.04**	**4.831**
Seeds order x Seeds invigoration treatments				
PCO	**11.17**	**71.33**	**102.3**	**81.87**
PB	**12.00**	**81.32**	**134.7**	**107.7**
PG	**11.00**	**74.34**	**121.7**	**97.33**
PBG	**12.66**	**88.00**	**157.7**	**126.1**
SC0	**11.00**	**68.67**	**100.0**	**80.00**
SB	**12.33**	**77.33**	**128.0**	**102.4**
SG	**10.66**	**72.00**	**115.0**	**92.00**
SBG	**12.33**	**81.68**	**151.0**	**120.8**
TCO	**9.33**	**59.00**	**75.70**	**60.53**
TB	**9.33**	**65.00**	**83.00**	**66.40**
TG	**9.00**	**65.00**	**72.30**	**57.87**
TBG	**10.33**	**68.00**	**86.7**	**66.40**
LSD 0.05	**0.716**	**5.343**	**9.46**	**7.568**

It could be observed from research results that seed order of carrot plant significantly impacted the production quality and quantity. In fact; the more order number increase; the less seed quality obtained (30, 37). However; seeds invigoration treatments ameliorated this situation by affecting multiple levels. It is evident that receiving gamma ray in low doses triggers morpho-development cascade that changes gene expression and protein or metabolite accumulation (14, 45).our results are consistent with this fact and that was obviously clear by observing figure (1) which illustrates that carrot seeds came from various seeds order showed rapid response in endo-β-mannanase enzyme activity (the enzyme that highly correlated with healthy germination and seedling establishment) (12, 21) and that reflected on plant performance and vigorousness in the field. However; the most satisfied findings were obtained from first and second orders seeds. The seeds of tertiary umbels had extremely subpar field performance even with the seeds that subjected to gamma ray. Seeds treated with *Tricoderma asperellum +Glomus intraradices* exhibited high standard performance in field traits because of the earliness of inoculation that provided all the advantages of biofertilizers from the first face of germination until plant harvest, that's mean: more solubility of nutrients, more pathogens combating, and more promoting hormones; organic acids secretion (1, 10, 19, 31). And that all created healthy and sustainable environment supported plant fitness. The maximum obtained benefit was from invigorating first and second order seeds with both of gamma ray and bio inoculants which influenced plant field performance in a large scale; the combination of both advantages could be used to interpret this. In conclusion; it would be advocated for the utilization of seed invigoration technology due to its minimized environmental, affordability, and ability to conserve resources.

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