ROLE OF FERMENTATION PRODUCTS ON DETERIORATION DATE PALM TREES INFECTION BY RED WEEVIL PEST

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Abstract. Date palm plants (*Phoenix dactylifera* L.) are a significant fruit grown worldwide under the biotic stress of red weevil pest (RWP) and microbial communities associated. Fermentation process in date palm trunk regarding infestation by RWP, yielding order and oozes, is the main diagnostic factor. These syndromes, related to activities of secondary infection by opportunic communities of bacteria, fungi and yeasts, which secrete volatile toxic materials, can suppress the growth of roots and apical meristem, as well as predispose date plants to root rot symptoms. The GC-MS analysis on the sample of fermented tissue of date palm infested by RWP yielded several volatile chemical components that phytotoxicity activities of seed germination of date palm and present its for development growth at seedlings stage such as ethyl alcohol, ethyl propanoate, limonene and linalool, these volatile were spread to apical meristem causing rotten of upper leaf base and down to induce root rot disease symptoms. So, the new strategy for suppressive degradable microbial communities and their secondary toxic materials secretion in the trunks of date palm trees is very important for reducing deterioration associated with the red weevil pest.

Keywords: deterioration; date palm; red weevil pest root rot; degradation.

INTRODUCTION

Date palm trees (*Phoenix dactylifera* L.) are grown worldwide in tropical and subtropical countries, in millions of hectares cultivated with more than 100 million date trees [3]. Date fruit, a vital nutrients of human, high reach of sugar as carbohydrates, 44-80%, protein, 2.3-5.6% and dietary fiber 6.4-12%, water, 15-30%, mineral of potassium, iron, calcium and low of sodium and fat, flesh dates contains 0.2-0.5% oil, whereas the seed contains 7.7-9.7% oil [4]. In addition, date fruits have a high content of phenolic compounds, including antimicrobial, antitumor, and antioxidant properties, making them a good source of diet, food and medical industrial products [2, 8, 38]. Red weevil pest (RWP), *Rhynchophorus ferrugineus* (Olivier), causing severe damage to dates would lead to the death of palm tree within 6-8 months [28], with no insecticide, no methods, no agents at any country have been able to eliminate the weevils [13, 16, 20, 23, 37]. Several volatile organic chemicals are phytotoxic to plant growth, i.e., acetic acid has a greater inhibitory effect on barley roots and shoot growth at the seedling stage [17]. During anaerobic conditions ethanol are produced which accumulated and inhibited amylase enzymes and development growth of plant tissue of rice seedling and cereal seeds [12]. The harmful effect of application exogenous ethanol on carrot tissue regarding to production acetaldehyde from ethanol by enzyme of alcohl dehydrogenase Ec1.1.1.1 [31]. Production volatile organic acid (VOA) of ethanol, acetic acid and ethyl acetate during generated of compost responsible for phytotoxicity of plant growth in date palm trunk tissue infested by red palm weevil [27]. Ethanol, lactic acid, carbon dioxide and hydrogen gas were produced of fruit of date palm [15]. In this manner, from date palm leaves other biological active chemical were isolated by TLC analysis of flavonols and flavones showed antifungal activity against pathogenic fungi *F. oxysporum* f. sp. *albedinis* (FOa) [7]. Several degradable bacteria and fungi were associated with RWP infestation of dates showed synergistic effect for deterioration of dates [40, 41]. This investigation aimed to study the roles of volatile components on the deterioration of date palm trees infected by red weevil pest.

MATERIALS AND METHODS

Total count of date palm trunks infected with Red weevil pest (RWP)

One g. of fermented tissue from different parts of the date palm trunk Cv. Zaghlol infested by red weevil pest in various stages of fermentation were diluted in 99 mL of sterilized distilled water in Erlenmeyer Flask 250 mL. Flasks were shaken for 30 minutes. Dilutions of each sample suspension were prepared at 1/10000. One mL of each dilution put on Petri dish then agar media were poured *i.e*., Cetrimide agar medium as a selective medium for counting fluorescent *Pseudomonas* bacteria [10]. Martin agar medium suplemented with streptomycin sulfate used for fungal isolation and yeast peptone sucrose agar medium for yeast amended by sodium propionate and chloraphenecol. Plates were incubated at 28 °C for 2-5 days for counting bacteria and fungi respectively according to the cultural characterizations [9, 11, 29].

Effect of the extraction of fermented trunk tissue on the germination of date palm seeds

Water extraction from the fermented tissue of date palm trunks infected by the red weevil was prepared. One Kg of fermented tissue was suspended on two litre of sterilized distilled water then shaking for 30 minutes then filtered through three layers of clotheeth. Different concentration were prepared, *i.e.,* 0, 25, 50 and 100%. Date palm seeds of Cv. Zaghloul were germinated after surface sterilized on wetted filter paper by 100 mL of each concentration of extraction in Petri- dishes (18-cm

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in diameter) incubated at 30 ºC. for one week. Ten seeds were suspended in each concentration on wetted filter papers, five plates were used as replicates. Seeds in five plates were treated with sterilized water as a control. Petri dishes were incubated at 35 ± 2 °C for 15 days in dark incubation conditions. The percentage of rotten and germination percentage on date palm was estimated according to [40].

HS-SPME Extraction of fermentation tissue of dates

A weight of 50 g of the date trunk fermented sample was used for the HS-SPME method of extraction of the volatile components. The sample was introduced into a 250 mL glass conical flask closed with parafilm and aluminium foil, and a Supelco SPME device, coated with polydimethylsiloxane (PDMS, 100 µm), was inserted into the flask. The system was left for 30 min. in the water bath at 50 ºC to allow equilibration. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC and GC–MS system.

GCMS analyses of volatile

The GC-MS analysis of the essential volatiles was carried out using gas chromatography-mass spectrometry with the following specifications: a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-5MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Analyses were performed using helium as the carrier gas at a flow rate of 1.0 mL/min. And a split ratio of 1:10 using the following temperature program: 60 ºC for 1 min.; rising at 3.0 ºC / min. to 240 ºC and held for 1 min. The injector and detector were held at 240 ºC. The sample injection was carried out manually. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Statistical analysis

Data obtained was statistically analysed using Duncan's multiple range test according to Snedecor and Cochran [36].

RESULTS

Count of microbes associated with date palm trunk infested by RWP

Data in Table 1 indicated that the total count of bacteria, fungi and yeast were counted in various infested stage of date palm by red weevil pest as shown in Fig. 1 i.e in trunk tissue intially infestation on leaf base with slight lytic, moderate infested degree with the a semi fermentation stage which recorded high lytic of tissue and the finally stage its completely fermentation with severly lysis of tissue in trunk. Data

Table 1. Frequency of microbial on date palm infested by red weevil pest

Samples of trunk fermented tissue	Count $x10^4$ Bacteria	Yeast	Fungi	Frequency% of fungi on date palm *A. niger*	*Fusarium* spp.	*Penicillium* spp.
Initial stage	98.0 a	192.0 a	11.0 b	0.0 b	100 a	0.0 b
Semi stage	36.0 b	58.0 b	16.0 a	31.3 a	0.0 b	68.8 a
Final stage	2.0 c	29.0 c	0.0 c	0.0 b	0.0 b	0.0 b

Values followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test

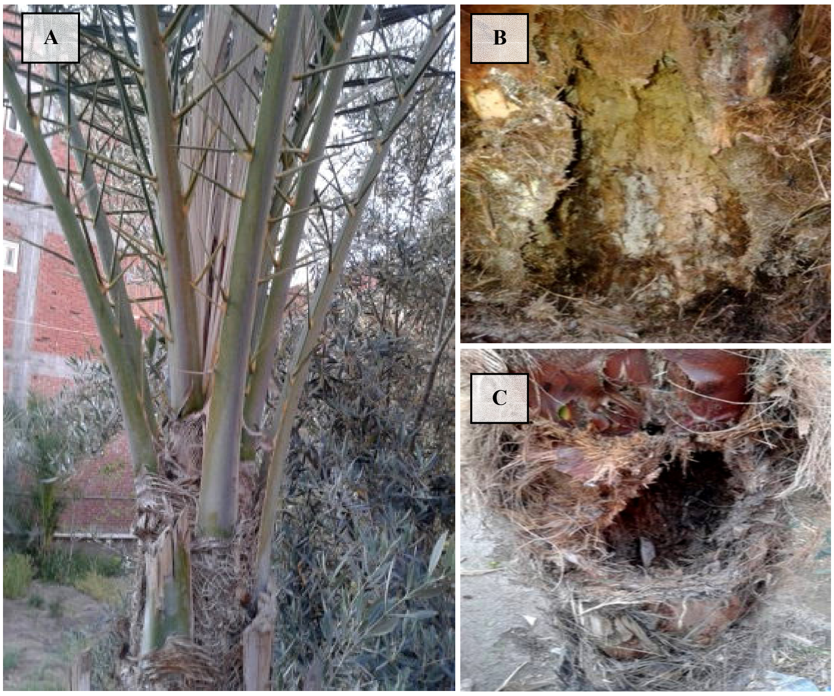

Figure 1. Date palm infested with RWP, showing wilt and dry apical growth (A), fermentation tissue (B) and (C) tunnel formation in the trunk of the date palm tree.

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indicated the microbial count of bacteria, fungi, and yeast counts were higher at initial fermentation of date palm trunk, with high counts of yeasts than bacteria. The count of bacteria gradually decreased with a moderate count at the semi-fermentation stage, then the lowest count at the final complete stage. In general, at all fermentation stages, the higher count of yeasts followed by the count of bacteria, while the fungi count was the lowest. Three fungal genera were detected with high frequency of *Fusarium* spp., at the initial fermentation stage only, meanwhile *Aspergillus niger* and *Penicillium* spp. were observed only at the semi-fermentation stage. Meanwhile, no observation of fungal count in the final fermentation stage.

Effect of date palm extraction on fermented trunk tissue on the rotten of date palm

Data in Table 2 indicated that soaking germinated seeds of date palm in various concentrations of extraction from fermented tissue of date palm trunk infection by red weevil pest, i.e., 25, 50 and 100%, caused root rot symptoms compared to the control. Increasing the concentrations of the extract increased the root rot incidence of date palm germinated seeds. Different root rot syndromes were observed, i.e., softening, maceration of root tissue and dark brown discolouration as shown in Figure 2.

Table 2. Effect of date palm extraction of fermented tissue on root rot incidence on date palm

Extraction % of fermented tissue	Root rot incidence on seed germination of date palm%
0	00.0d
25	10.0c
50	20.0b
100	50.0a

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Figure 2. Effect of different concentrations of fermented extraction, 1= 25%, 2= 50% and 3= 100%, on the germination of date palm seeds. Normal growth (0) showing root and stem growth. Abnormal rapid growth, 1, 2 and 3 showed the absence of a stem, abnormal elongation and more thickness of the root and maceration with brown discolouration compared to the normal germination of the seed in the control (**0=** distilled water).

Chemical components in fermented date palm tissue infected with RWP

The chromatogram of the main volatile compounds extracted by the HS-SPME technique and separated by GC-MS is shown in Fig. 3. The retention time (Rt..) in minutes and the relative area percentages of the main volatile components are shown in Table 3. The primary compound was identified as ethyl butanoate, with a relative percentage of 46.64% of the total identified components. The second main compound was identified as iso-amyl n-butyrate with 11.33% of the total identified compounds, followed by p-Mentha-1,3-diene (6.8%), then L-Limonene (6.09%). Ethyl caproate, terpinolene, ethyl acetate, and ethyl alcohol were identified and accounted for relative area percentages of 5.97, 5.07, 4.14, and 2.91%, respectively. Amyl butyrate (1.78%), isoamyl propionate (1.67%), isobutyl butanoate (1.43%), and ethyl propanoate (1.00%) were also identified in Fig. 2 and Table 2.

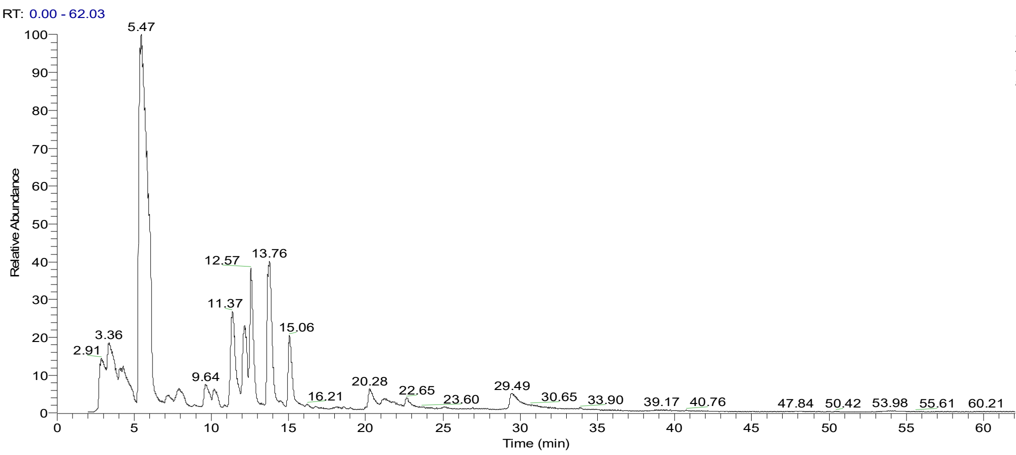

Figure 3. GC-MS chromatogram of the main volatile compounds extracted by HS-SPME technique

Table 3. The main volatile components extracted by SPEM of the infected palm hart as separated and identified by GC-MS.

Peak No.	RT (min.)	Area %	Compound	Chemical formula
0	1	2	3	4
1	2.91	2.91	Ethyl alcohol	C_2H_6O
2	3.36	4.14	Ethyl acetate	$C_4H_8O_2$
3	4.32	1.00	Ethyl proanoate	$C_5H_{10}O_2$
4	5.47	46.64	Ethyl butanoate	$C_6H_{12}O_2$

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0	1	2	3	4
5	7.21	0.78	Isoamyl acetate	$C_7H_{14}O_2$
6	7.93	1.78	Amyl butyrate	$C_9H_{18}O_2$
7	9.64	1.43	Isobutyl butanoate	$C_8H_{16}O_2$
8	10.24	1.67	Isoamyl propionate	$C_8H_{16}O_2$
9	10.84	0.10	Geranyl butyrate	$C_{14}H_{24}O_2$
10	11.37	5.97	Ethyl caproate	$C_8H_{16}O_2$
11	12.13	6.80	p-Mentha-1,3-diene	$C_{10}H_{16}$
12	12.57	6.09	L-Limonene	$C_{10}H_{16}$
13	13.76	11.33	Iso amyl n-butyrate	$C_9H_{18}O_2$
14	14.53	0.18	Linalool	$C_{10}H_{18}O$
15	15.06	5.07	Terpinolene	$C_{10}H_{16}$
16	16.21	0.13	Thujyl alcohol	$C_{10}H_{18}O$
17	18.59	0.12	trans-β-Terpineol	$C_{10}H_{18}O$
18	20.28	1.21	Ethyl caprylate	$C_{10}H_{20}O_2$
19	21.14	0.36	Estragole	$C_{10}H_{12}O$
20	29.439	1.16	Ethyl decanoate	$C_{12}H_{24}O_2$
Total		**98.87**		

Table 4. Phytotoxic effect of volatile chemical components

Component	Toxicity effect on plants	References
Ethyl alcohol (Ethanol)	stimulation or poison to parley plant when added to the nutrient solution in water culture and highly toxic on *Elodea* plant leaf by increasing permeability	[33]
	reduce floral scape and leaf elongation of 'Ziva' paperwhite narcissus (*Narcissus tazetta*) and reduced hight growth of peppermint schnapps flowers quality	[25]
	increase membrane permeability and damage lipid bilayer	[32]
	restricited root growth of rice seedlings	[12]
	progressively reduce the number of root on *Phaseolus aureus*	[24]
	ethanol phytotoxic date palm growth	[27]
Ethyl acetate	suppress growth of date palm plants	[27]
Ethyl propanoate	effect on growth and enzyme activities of *Zoysia martellas*	[18]
Limonene	significantly phytotoxic cabbage and carrot	[19]
	suppress germination and growth of *Lactuca sativa* and *Allium cepa*	[34]
	completely inhibition seed germination of *Lolium rigidum*, *Sinapis arvensis*, and *Raphanus raphanistrum* , inhibited growth of *Lacta sativa*	[5]
Linalool	reduce germination, growth of hypocotyl and radicles in *Majorana hortensis*, *Trifolium repens*, *Rudbeckia hirta*, *Chrysanthemum zawadskii*, *Melissa officinalis*, *Taraxacum platycarpum*, and *Tagetes patula*	[21]

Phytotoxic effect of volatile chemical components

Phytotoxicity effect of four volatile components on plant tissue wa fixed in literature as shown in Table 4, ethyl alcohol, ethyl propanoate, ethyl acetate, limonene and linalool were adverse effect on plant growth, physiological reaction, enzyme activities, cell permeability, damage lipid bilayer, number of root on rice seedlings, plant of *Phaseolus aureus*, pepper white, *Zoysia martellas* and *Elodea* sp.

DISCUSSION

The red weevil pest (RWP), *Rhynchophorus ferrugineus* (Olivier) is a large polyphagous insect native to Southern Asia then widely distributed every country of Asia, Europe, Africa, and American [39]. Red palm weevil (RWP) is a concealed tissue borer, and all stages of its life cycle are found inside the trunk of the date palm tree, consuming the tender soft tissues. The most view symptoms of palm trees are presence of tunnels in trunk, oozing of thick yellow to brown fluid from date trees, appearance of chewed plant tissues in and around openings tunnels in the trunk, presence of a fermented odor from the fluid inside infested tunnels in the trunk, and/or breaking of the trunk or toppling of the crown [20]. Several bacteria and fungi associated with date palm infested by red weevil pest can induce lysis and pathological syndromes of date palm tissue such as *Pseudomonas aurgenosa* [40] and species of fungal genera of *Aspergillus* spp., *Botryodiplodia theobromae, Fusarium* spp., *Rhizoctonia solani* and *Fusarium oxysporum* were the most frequent fungal genera caused roten of germinated seeds and seedlings of date palm as well as and *F. oxysporum* f. sp. *albedinis (FOa)* is one of the major soil-borne pathogens that induces vascular wilt on date palm [6, 7, 41] . In this study, water extraction of fermented trunk tissue significantly caused root rot syndromes of date palm germinated seed, which increased with increasing concentration of toxic materials secreted during the germination process. The deterioration of date palm trunk infested with red weevil increased due to the synergistic effect of the secondary infection of fungi, which could produce secondary metabolites of volatile toxic materials, negatively affecting the growth of plant roots and seedlings [15, 17, 27]. In this manner, the GC-MS chromatogram of fermented sample from date palm trunk infested by (RWP) indicated that several volatile compounds were extracted and identified as ethyl butanoate, iso-amyl n-butyrate, p-mentha-1,3-diene, L-limonene, ethyl caproate, terpinolene, ethyl acetate, ethyl alcohol, amyl butyrate, isoamyl propionate, isobutyl butanoate, and ethyl propanoate. In this respect, these components are reported as toxic plant growth. In this respect, ethanol, acetic acid and acyl acetate are degraded during the fermentation of

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palm tissues infected by the red palm weevil pest, *Rhynchophorus ferrugineus*, which suppresses nematode biocontrol agents, *Heterorhabditis indica*, against RWP [27]. Ethanol was highly toxic which increasing permeability on Elodea leaf [33], ethanol significantly inhibited number of root of (*Phaseolus aureas*) which increased by increasing their concentration [24], ethanol increase membrane permeability and damage lipid bilayer [32], root-zone treatment with different sources of ethanol at 4% to 5% (v/v) were reduced hight growth of peppermint schnapps flowers quality [25]. Ethyl butanoate (ethyl butyrate) is a volatile ethyl ester found in alcoholic beverages, found in many fruits and cheese at lower concentrations,its produced during fermentation by yeast and formed by the reaction between ethanol and butyric acid [30], also, its producing by several bacterial genera of *Streptococcus* and *Lactococcus* [22]. Ethyl butanoate obtained via esterification of butanoic acid and ethyl alcohol in the presence of *Candida antarctica* [26], additionally, it's also the main active component of volatiloma, a soil fumigant agent against nematode, *Meloidogyne javanica*, which is extracted from *Cassia waste* [14]. Isoamyl acetate, produced via fermentation by alcohol acetyl-transferases from *Saccharomyces cerevisiae* [35]. Limonene is one of the main biological constituents of essential oils at 90 and 120 mL/L were significantly phytotoxic in both cabbage and carrot by caused leaf injuries, reduced vegetative growth, chlorophyll and photosynthesis [19], limonene of *Heterothalamus psiadioides* essential oil inhibiting germination, growth of shoots and roots on *Lactuca sativa* and *Allium cepa* [34]. Limonene of *Pinus pinea* EO inhibits *Lolium rigidum, Sinapis arvensis*, and *Raphanus raphanistrum*, and seed germination is completely inhibited at low concentrations [5]. In addition, linalool EOs of *Agastache rugosa* completely prevented germination and growth of hypocotyl and radicles in *Majorana hortensis*, *Trifolium repens*, *Rudbeckia hirta*, *Chrysanthemum zawadskii*, *Melissa officinalis*, *Taraxacum platycarpum*, and *Tagetes patula* [21]. Linalool high phytotoxicity in the EOs of many plants [1]. So, the new strategy must be concerned with inhibiting degradable microbial communities' activity of the root and trunk of date palm trees to suppress the production of volatile toxic materials, which increases deterioration by the red weevil pest on date palm.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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