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Biocontrol potential of *Saccharomyces* as a sustainable approach targeting *Spodoptera frugiperda*

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***Spodoptera frugiperda*, known as the fall armyworm, is a highly destructive pest that greatly impacts worldwide agriculture, particularly maize and other key crops. Its rapid expansion and resistance to standard insecticides pose a significant threat to food security. As a result, effective and long-term pest management solutions are required. In this context, microbial biocontrol agents, such as *Saccharomyces cerevisiae*, offer an environmentally friendly alternative to chemical pesticides. This study investigates *S. cerevisiae*'s efficiency as a biocontrol agent against *S. frugiperda*. The *S. cerevisiae* HA-NY4 strain was evaluated for larvicidal activity as well as its effect on insect metabolism and development. The results showed that *S. cerevisiae* HA-NY4 significantly increased larval mortality after 72 h of treatment. Furthermore, it caused metabolic abnormalities in the larva, including reduced protein synthesis, impaired carbohydrate metabolism, and developmental defects. These effects resulted in delayed pupation, lower pupal weight, and decreased adult emergence, thereby restricting the pest's reproductive potential. The main goal of this study was to evaluate the effectiveness of the *S. cerevisiae* HA-NY4 strain, thus providing a sustainable and environmentally friendly solution to manage *S. frugiperda* infestations.**

Keywords Biocontrol, Chitinase, Entomopathogens, Invertase, *Saccharomyces cerevisiae*, *Spodoptera frugiperda*

Abbreviations

AMY	α-Amylase enzyme
CHI	Chitinase enzyme
INV	Invertase enzyme
IPM	Integrated pest management
L-long	Larval longevity
P-long	Pupal longevity
P-wt	Pupal weight
TRE	Trehalase enzyme
S. cerevisiae	*Saccharomyces cerevisiae*
S. frugiperda	*Spodoptera frugiperda*
S. uvrum	*Saccharomyces uvrum*

Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae), called the fall armyworm, is a destructive pest mainly of maize (corn), although it can attack a variety of other cereal crops and vegetables[1]. Having been introduced from the Americas, it has since dispersed to different areas of the world, such as Africa, Asia, and Europe[2]. This polyphagous and migratory pest attacks mainly maize, but it also infests rice, sorghum, and many vegetables and causes considerable economic losses worldwide[3]. It has four stages before it is completely a butterfly: egg, larva (caterpillar), pupa, and adult[4]. The larvae during this destructive phase are extremely active and voracious, feeding on plant tissues and resulting in severe damage to the leaves, stems, and reproductive

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organs[5]. The fall armyworm has six larval instars, and it is the fourth to sixth instars that cause much damage to a plant. Yet, the egg and pupal predation have been poorly investigated in the contemporary biocontrol practices[6]. In endemic areas, fall armyworm populations often show cross-resistance to several classes of the insecticides used due to prolonged exposure to traditional insecticides[7]. Economic consequences include losses beyond direct losses in yield; farmers often spend more to apply pesticides and other controls to control this pest[8,9].

Microbial insecticides also provide promising impacts in managing the fall armyworm population[10]. Many genera of entomopathogenic fungi, including *Beauvaria*, *Trichoderma*, and *Nomuraea rileyi*, proved to be promising agents against fall armyworms. These fungi are capable of targeting and killing *S. frugiperda* larvae through different infection processes[11]. In worldwide studies, *Metarhizium rileyi*, *Beauveria bassiana*, and *Bacillus thuringiensis* are regarded as the best-studied entomopathogens with established field efficacy against fall armyworm larvae. Nevertheless, efficacy rates vary greatly because of organismal and geographic factors, and the field must strive for a broader screening of microbial agents, such as yeast-based interventions[12]. Microbes provide an environmentally friendly and sustainable solution for pest control below the economic injury threshold while enhancing overall plant vitality and yield. Entomopathogens, which are microorganisms capable of infecting and eliminating insects, have been investigated as a sustainable substitute for chemical insecticides in pest management[13]. While much of the research has concentrated on bacteria, fungi, and viruses, there have been studies examining the viability of yeast as biocontrol agents[14].

The genus *Saccharomyces* is a genus of yeast generally used in a bioprocess, such as food fermentation, or various industrial processes processes[15]. Although *Saccharomyces* species are generally not considered entomopathogens, despite some strains of *Saccharomyces* have been studied as potential entomopathogenic agents[16]. *Saccharomyces cerevisiae* is a single-celled organism that researchers have studied extensively, and it is a powerful tool for studying eukaryotic organisms. *S. cerevisiae* (baker's yeast) has been applied in diverse industries such as food and beverage production[17]. It is a eukaryote that is single-celled, which makes many things easier to study since it performs most of the same biological functions as other eukaryotes. Additionally, it is simple to manipulate genetically. Unlike some other model organisms, *S. cerevisiae* holds significant importance in various biotechnological applications, some with a history spanning thousands of years[18]. *S. cerevisiae* is a probiotic yeast that is known to be safe for human health, making it a suitable biocide. Clinical studies acknowledged it as a biotherapeutic agent with antibacterial, antiviral, anti-carcinogenic, antioxidant, anti-inflammatory, and immune-modulating properties. Oral or intramuscular administration produces significant health-promoting benefits[19]. *S. cerevisiae* has been identified as a potential biological control of different kinds of plant pathogens and pests[20]. In addition, *S. cerevisiae* has demonstrated eco-friendly consequences in the environment by promoting plant growth and development and increasing plant resistance against abiotic stresses[21,22]. Moreover, *S. cerevisiae* can also activate systemic resistance in plants, increasing their resistance to numerous pathogens[23].

The overall goal of this study is to test *Saccharomyces*, entomopathogenic fungus, against the fall armyworm. We looked into its toxicity and biochemical investigations to figure out how *Saccharomyces* works as an alternative biopesticide. Our work highlights the importance of environmentally friendly entomopathogens in pest management practices.

Results

Bioassay of *Saccharomyces* strains' treatments

At 48 h post-treatment, both *Saccharomyces* strains showed limited larval mortality activity against *S. frugiperda*. *S. cerevisiae* HA-NY4 had a mortality rate of 0.67 ± 0.47, while *S. uvrum* HA-NY3 and the control groups (control and control media) showed no mortality. Diazinon 60% EC, a commercial pesticide, produced no noticeable mortality at this time point, consistent with its method of action, which normally requires a longer duration to cause adverse effects, as illustrated in Table 1. The p-value from the ANOVA indicates that there is no significant difference between the treatments ($p > 0.05$). This indicates that after 48 h, neither *Saccharomyces* strains nor Diazinon 60% EC had a significant effect on larval mortality.

At 72 h post-treatment, major changes begin to emerge *S. cerevisiae* HA-NY4 showed higher larvicidal activity, with the number killed per day, a mortality rate of 1.33 ± 0.47, while *S. uvrum* HA-NY3 had no effect on larvae. Diazinon 60% EC had a somewhat higher rate of 2.00 ± 0.00 than both *Saccharomyces* strains. The control groups had no mortality, as illustrated in Table 1. The one-way ANOVA statistical analysis shows that treatments had a significant impact on mortality rates at 72 h post-treatment ($p < 0.05$). Tukey's HSD shows that *S. cerevisiae* HA-NY4 was significantly more effective than the control groups but less effective than Diazinon 60% EC.

The mortality rates observed at 120 h add further insight into the long-term effectiveness of the treatment. No additional direct mortality beyond that observed at 72 h occurred with *S. cerevisiae* HA-NY4. *S. uvrum* HA-NY3 produced a delayed toxic effect, with a mortality of 0.33 ± 0.47. Diazinon 60% EC reduced mortality to 1.00 ± 0.82. The ANOVA analysis shows no significant differences between treatments ($p > 0.05$), meaning that 120 h was sufficient to minimize the significant differences seen at 72 h. For instance, *S. uvrum* HA-NY3 displayed low toxicity (after 120 h), likely due to its superior efficacy (*S. cerevisiae* HA-NY4 and Diazinon 60% EC being comparable). Figure 1 demonstrated some direct toxic effects of *Saccharomyces* strains on treated *S. frugiperda* fourth instar larvae.

Biological aspects of *Saccharomyces* strains' treatments

As exhibited in Table 2, the biological alterations resulting from *S. frugiperda* larvae's treatment with various *Saccharomyces* strains provided novel perspectives into the undocumented specific interactions of these biocontrol agents. Those were *S. cerevisiae* HA-NY4, *S. uvrum* HA-NY3, the commercial pesticide Diazinon 60% EC, and controls. Our study covered a variety of essential biological parameters, including larval and pupal survival, pupal weight, the occurrence of deformations, and the number of freshly emerging adults. Figure 2

Treatments	Toxicity rate 48 h posttreatment	Toxicity rate 72 h posttreatment	Toxicity rate 120 h posttreatment
Control	00.0 ± 0.00 b	00.0 ± 0.00 c	00.0 ± 0.00
Control media	00.0 ± 0.00 b	00.0 ± 0.00 c	00.0 ± 0.00
Diazinon 60% EC	00.0 ± 0.00 b	2.00 ± 0.00 a	1.00 ± 0.82
S. cerevisiae HA-NY4	0.67 ± 0.47 a	1.33 ± 0.47 b	00.0 ± 0.00
S. uvrum HA-NY3	00.0 ± 0.00 b	00.0 ± 0.00 c	0.33 ± 0.47
ANOVA: p-value	0.009**	< 0.001***	0.063 ns

Table 1. Mortality of *S. frugiperda* larvae to tested *Saccharomyces* strains. Data are expressed as mean ± SD; *, **, and *** are significant at $p < 0.05$, < 0.01, and < 0.001, respectively; ns, non-significant at $p > 0.05$. The mean under each variety with different letters in the same column (vertically) denotes a significant difference according to Tukey's HSD at $p \leq 0.05$. Control, control *S. frugiperda* larvae treated with distilled water for experiment adjustment; Control media, control *S. frugiperda* larvae treated with yeast extract peptone dextrose broth medium for experiment adjustment; Diazinon 60% EC, *S. frugiperda* larvae treated with commercial pesticide Diazinon 60% EC as a positive control; *S. cerevisiae* HA-NY4, *S. frugiperda* larvae treated with *S. cerevisiae* HA-NY4 strain; *S. uvrum* HA-NY3, *S. frugiperda* larvae treated with *S. uvrum* HA-NY4 strain. We used Diazinon 60% EC at its recommended rate of 1 mL/L. All bioassay assessments were triplicated (under the same conditions).

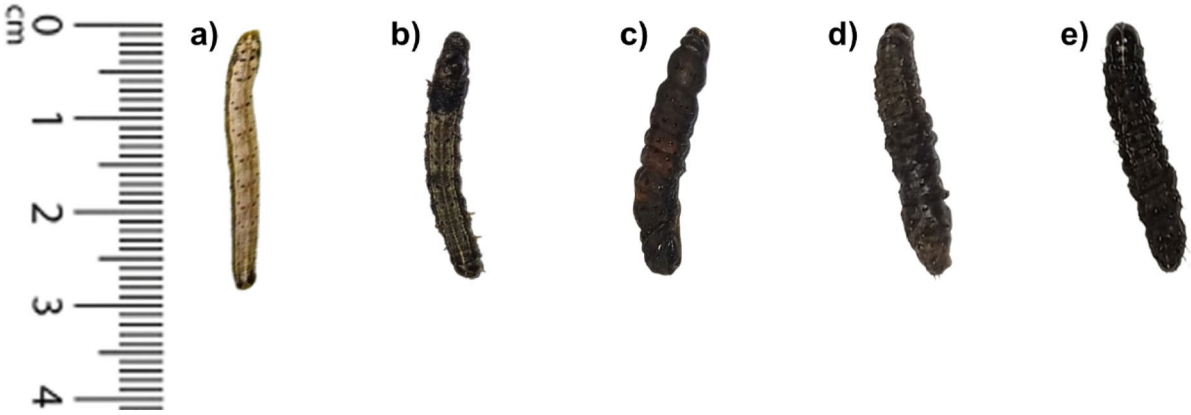

Fig. 1. Direct toxic effects of *Saccharomyces* strains on *S. frugiperda* fourth instar larvae. (**a**), control *S. frugiperda* larvae; (**b**), dead *S. frugiperda* larvae treated with *S. uvrum* HA-NY3 48 h post-treatment; (**c**,**d**), dead *S. frugiperda* larvae treated with *S. cerevisiae* HA-NY4 48 h post-treatment; (**e**), dead *S. frugiperda* larvae treated with Diazinon 60% EC 48 h post-treatment.

Treatments	Larval longevity (days)	Pupal longevity (days)	Pupal weights (g)	Occurrence of deformations	Newly emerged adults
Control	6.67 ± 0.47 c	8.00 ± 0.00 c	0.15 ± 0.01 a	00.00 ± 00.00 d	8.00 ± 0.00 a
Control media	6.67 ± 0.47 c	8.00 ± 0.00 c	0.16 ± 0.02 a	00.00 ± 00.00 d	8.00 ± 0.00 a
Diazinon 60% EC	10.00 ± 0.82 a	9.00 ± 1.41 ab	0.15 ± 0.01 a	0.67 ± 0.47 c	4.33 ± 0.47 b
S. cerevisiae HA-NY4	9.67 ± 0.47 ab	9.00 ± 0.00 ab	0.13 ± 0.01 a	1.00 ± 0.82 b	4.33 ± 0.94 b
S. uvrum HA-NY3	9.00 ± 0.00 b	9.33 ± 0.47 a	0.15 ± 0.01 a	2.33 ± 1.25 a	5.33 ± 1.25 b
ANOVA: p-value	< 0.001***	0.098 ns	0.204 ns	0.012*	< 0.001***

Table 2. Biological aspects of *S. frugiperda* larvae to the tested *Saccharomyces* strains. Data are expressed as mean ± SD; *, **, and *** significant at $p < 0.05$, < 0.01, and < 0.001, respectively; ns, non-significant at $p > 0.05$. The mean under each variety with different letters in the same column (vertically) denotes a significant difference according to Tukey's HSD at $p \leq 0.05$. Control, control treated with distilled water for experiment adjustment; Control media, control treated with yeast extract peptone dextrose broth medium for experiment adjustment; Diazinon 60% EC, *S. frugiperda* larvae treated with commercial pesticide Diazinon 60% EC as a positive control; *S. cerevisiae* HA-NY4, *S. frugiperda* larvae treated with *S. cerevisiae* HA-NY4 strain; *S. uvrum* HA-NY3, *S. frugiperda* larvae treated with *S. uvrum* HA-NY4 strain. Diazinon 60% EC was used at its recommended rate of 1 mL/L. All bioassay assessments were triplicated (at the same conditions).

illustrates the latent effects of *Saccharomyces* strains on the growth and survivability of treated *S. frugiperda* fourth-instar larvae, revealing visible evidence of larval and pupal mortality and deformations. Table 2 in the comparison of treated groups with control groups, a significant increase in larval longevity was observed in those which received Diazinon 60% EC and *Saccharomyces* strains. The larval longevity was 9.67 ± 0.47 days for larvae treated with *S. cerevisiae* HA-NY4, and for the Diazinon 60% EC treatment, it was 10.00 ± 0.82 days. In comparison, control groups had much shorter larval longevity of 6.67 ± 0.47 days ($p < 0.001$), indicating faster maturation of untreated larvae, consistent with optimized conditions.

On the other hand, pupal longevity varied significantly between treatments. Control larvae pupated in 8 days in both control groups. In contrast, larvae exposed to Diazinon 60% EC had a pupal longevity of 9.00 ± 1.41 days, whereas those treated with *S. uvrum* HA-NY3 had a significantly longer pupal duration of 9.33 ± 0.47 days. The *S. cerevisiae* HA-NY4 treatment group showed the most significant effect, with pupal longevity remaining 9 days. The observed developmental delay, particularly in the *S. cerevisiae* HA-NY4 group, underscores the strain's potential as a biological control agent, exhibiting effects similar to those of chemical pesticides.

Pupal weight is an important element that influences the fitness and reproductive potential of adult moths. Table 2 shows a decrease in pupal weights in all treatment groups when compared to the control. The average pupal weight in the control groups was 0.15 ± 0.01 g for control treated with distilled water and 0.16 ± 0.02 g for control treated with yeast extract peptone dextrose broth medium. The Diazinon 60% EC treatment resulted in a slight reduction in pupal weight (0.15 ± 0.01 g), while *S. cerevisiae* HA-NY4 and *S. uvrum* HA-NY3 treatments resulted in considerable decreases (0.13 ± 0.01 g and 0.15 ± 0.01 g, respectively).

Moreover, Table 2 shows that the incidence of deformations increased significantly in the treated groups. The control groups showed no deformations. However, the *S. uvrum* HA-NY3 treatment had a high frequency of deformations (2.33 ± 1.25), whereas the *S. cerevisiae* HA-NY4 group had moderate deformation rates (1.00 ± 0.82). Diazinon 60% EC's treatment led to minor deformations (0.67 ± 0.47) compared to the *S. uvrum* HA-NY3 group. Figure 2 shows that the treatment with *S. cerevisiae* HA-NY4 caused severe developmental abnormalities, particularly during the prepupal and pupal stages. Deformed larvae and pupae, shown in Fig. 2b, e, demonstrate *Saccharomyces*' negative impact on normal insect development.

The treatments have a major impact on adult development. All larvae in the control groups pupated and became adults in 8 days. Most importantly, all treatment groups showed a substantially reduced emergence of adults. The number of adults emerged was 4.33 ± 0.47 adults per group of Diazinon 60% EC, all of which had the

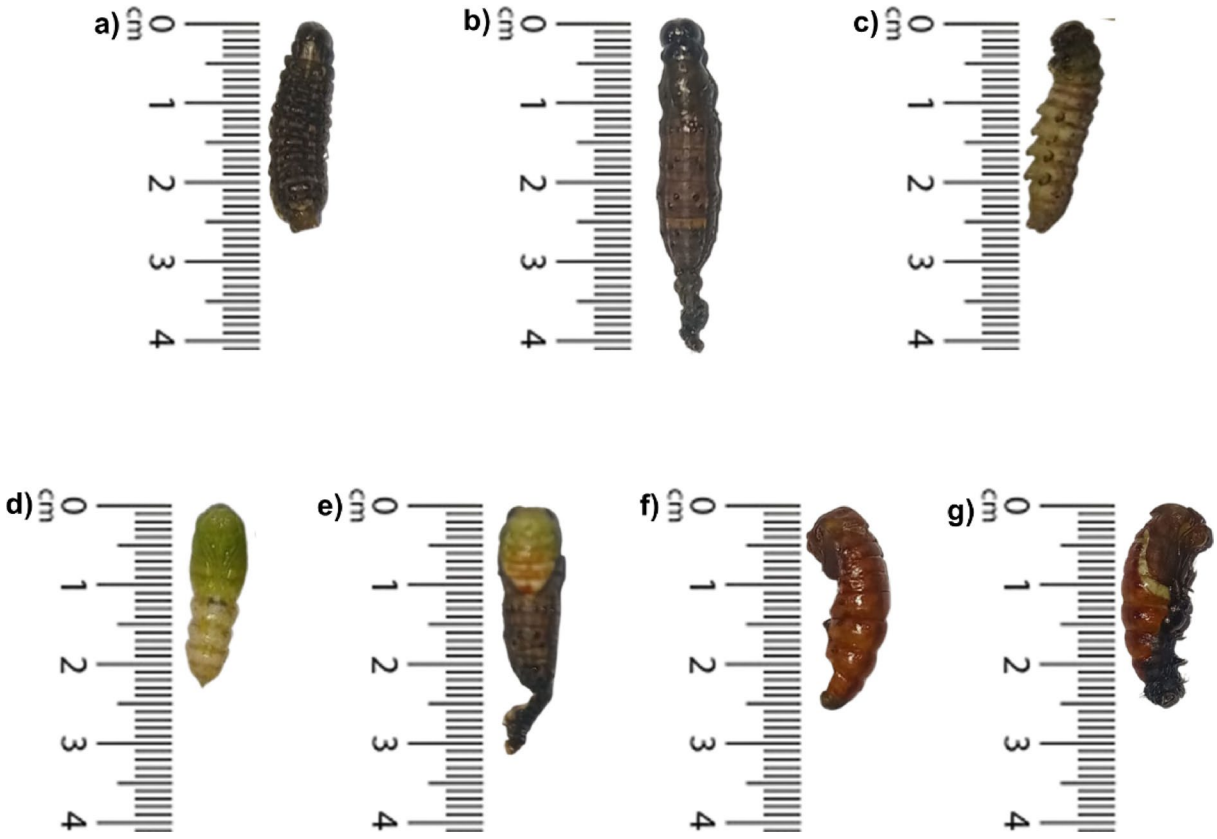

Fig. 2. Indirect "latent" toxic effects of *Saccharomyces* strains on *S. frugiperda* fourth instar larvae. (**a**), control *S. frugiperda* prepupae; (**b**), *S. frugiperda* six instar dead larvae treated with *S. cerevisiae* HA-NY4; (**c**), *S. frugiperda* dead prepupae treated with *S. uvrum* HA-NY3; (**d**), control fresh *S. frugiperda* pupae; (**e**), dead fresh *S. frugiperda* pupae treated with *S. cerevisiae* HA-NY4; (**f**), dead two-day *S. frugiperda* pupae treated with *S. cerevisiae* HA-NY4; (**g**), dead latent *S. frugiperda* pupae treated with *S. uvrum* HA-NY3.

same number emerged at 4.33 ± 0.94 adults per group of *S. cerevisiae* HA-NY4 (Table 2). Somewhat surprisingly, the *S. uvrum* HA-NY3 group showed the greatest reduction in adult emergence, with only 5.33 ± 1.25 emerging.

Biochemical assessments

Total soluble protein, total lipids, and total carbohydrates of treated S. frugiperda larvae

Total soluble protein concentration is a major indicator of protein metabolism in the larvae and the direct effects of treatments on such protein production or breakdown. When larvae were exposed to both *Saccharomyces* strains and Diazinon 60% EC, a significant alteration in total soluble protein levels was observed compared to the control groups (Fig. 3a). The protein concentration in control larvae subjected to distilled water was found to be 0.038 ± 0.0117 mg/g body weight, and those subjected to the control media were similarly less (0.032 ± 0.011 mg/g body weight). On the other hand, the Diazinon 60% EC treatment group demonstrated a higher protein concentration (0.056 ± 0.007 mg/g body weight), which demonstrates that the chemical insecticide also might have inhibited protein synthesis or activated either compensatory or increased protein retention mechanisms. In contrast, larvae treated with *S. cerevisiae* HA-NY4 decreased in protein content (0.056 ± 0.0297 mg/g body weight), whereas *S. uvrum* HA-NY3-treated larvae showed the greatest decrease (0.051 ± 0.0016 mg/g body weight).

Conversely, lipid metabolism regulation is strict in insects, and its impairment has a substantial impact on their survival. The total lipid in *S. frugiperda* larval body weight is helpful in estimating the effects of treatments on energy metabolism. Figure 3b shows that the control group treated with distilled water had a total lipid concentration of 41.91 ± 0.54 mg lipids/mg protein, whereas the control media group had slightly higher levels (42.83 ± 0.97 mg lipids/mg protein). The control groups' homogeneity suggests normal lipid metabolism in untreated larvae. Diazinon 60% EC treatment significantly increased total lipid content (87.59 ± 0.81 mg lipids/mg protein), indicating that the chemical pesticide disrupted normal lipid metabolism, perhaps leading to an accumulation of lipids. The *Saccharomyces* treatments have varying impacts on lipid metabolism. When larvae were treated with *S. cerevisiae* HA-NY4, there was a significant increase in lipid content (72.71 ± 0.39 mg lipids/

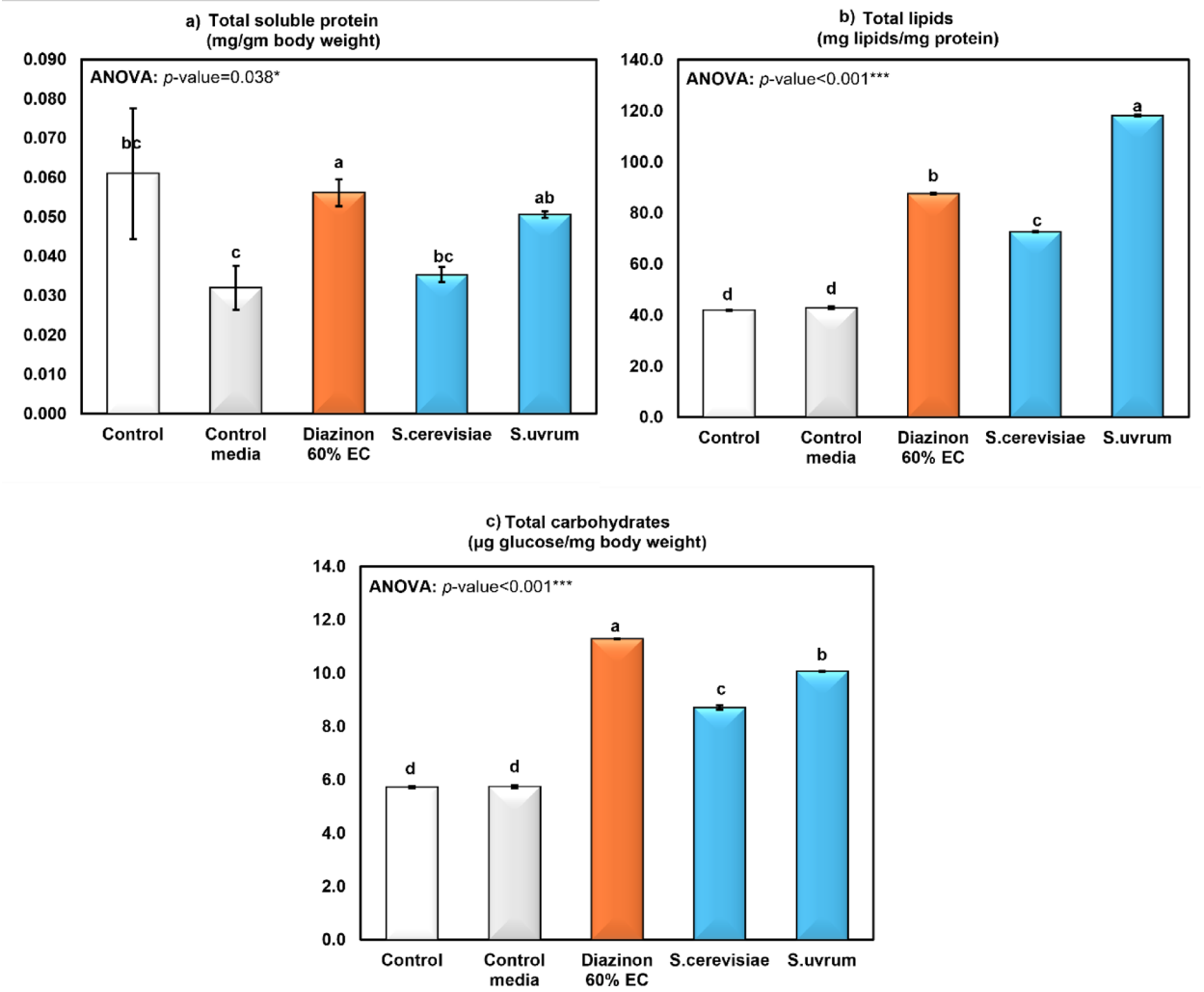

Fig. 3. Biochemical assessments of total soluble protein, total lipids, and total carbohydrates of *S. frugiperda* larvae treated with *Saccharomyces* strains.

mg protein), although not as much as when they were treated with Diazinon 60% EC. This increase suggests that *S. cerevisiae* HA-NY4 may either stop larvae from lipid utilization or cause them to store more lipids, similar to how the chemical pesticide works. The *S. uvrum* HA-NY3 treatment had the highest total lipid level (118.13 ± 0.67 mg lipids/mg protein), which means that the lipid metabolism in the larvae changed in a big way. *S. uvrum* HA-NY3 significantly affected lipid metabolism, which may provide a better selection of potential *S. uvrum* as biocontrol agents.

The total carbohydrate content of the larvae reveals the effects of the treatments on energy storage and utilization. The control group had a total carbohydrate content of 5.72 ± 0.076 µg glucose/mg protein, while the control media group that was treated with yeast extract peptone dextrose broth medium had somewhat higher carbohydrate levels of 5.74 ± 0.104 µg glucose/mg protein. These values correspond to baseline carbohydrate metabolism measured in untreated larvae. The carbohydrate content at the concentration of Diazinon 60% EC (11.278 ± 0.019 µg glucose/mg protein) was significantly higher. This means that chemical pesticides might have disturbed normal carbohydrate metabolism, causing an accumulation of glucose or glycogen. Likewise, the *Saccharomyces* treatments had increased carbohydrate levels versus the controls. The carbohydrate content was higher in larvae treated with *S. cerevisiae* HA-NY4 (8.71 ± 0.17 µg glucose/mg protein), and the group *S. uvrum* HA-NY3 showed an even higher carbohydrate level (10.067 ± 0.024 µg glucose/mg protein). These findings indicate that the two *Saccharomyces* strains have similar effects on carbohydrate metabolism in larvae, where glucose or glycogen accumulates.

The biochemical measurements (Fig. 3) indicate a marked decrease in protein, lipids, and carbohydrates metabolism in *S. frugiperda* larvae treated with different strains of *Saccharomyces* as well as with Diazinon 60% EC. *S. cerevisiae* HA-NY4 and *S. uvrum* HA-NY3 significantly affected these crucial biochemical indicators. This means that these strains interfere with larval energy consumption and protein synthesis. The substantial effects observed in the *S. uvrum* HA-NY3 treatment group, particularly lipid and carbohydrate accumulation, suggest that this strain may have a more potent biocontrol effect than *S. cerevisiae* HA-NY4.

Bars followed by different letters are significantly different according to Tukey's HSD at the 0.05 level.

Control, control *S. frugiperda* larval group treated with distilled water for experiment adjustment; Control media, control *S. frugiperda* larval group treated with yeast extract peptone dextrose broth medium for experiment adjustment; Diazinon 60% EC, *S. frugiperda* larval group treated with commercial pesticide Diazinon 60% EC as a positive control; *S. cerevisiae* HA-NY4, *S. frugiperda* larval group treated with *S. cerevisiae* HA-NY4 strain; *S. uvrum* HA-NY3, *S. frugiperda* larval group treated with *S. uvrum* HA-NY4 strain. We used Diazinon 60% EC at its recommended rate of 1 mL/L. All biochemical measurements were performed in triplicate.

Biochemical assays of AMY, INV, TRE, and CHI of treated S. frugiperda larvae

The enzymatic activities of α-amylase (AMY, EC 3.2.1.1), invertase (INV, EC 3.2.1.26), trehalase (TRE, EC 3.2.1.28), and chitinase (CHI, EC 3.2.1.14) enzymes were assessed using *S. frugiperda* larvae treated with the chemical pesticide Diazinon 60% EC, *S. cerevisiae* HA-NY4, and *S. uvrum* HA-NY3. These enzymes are required to digest nutritional macromolecules, and modifying activity in them should enable larvae to grow, develop, and survive. Figure 4 shows that larval metabolic enzyme activities were influenced by these treatments.

α-amylase (AMY, EC 3.2.1.1) is a crucial enzyme that assists in the digestion of carbohydrates, specifically starch. It promotes the hydrolysis of starch into maltose and glucose, both of which are essential for providing energy to the larvae. Amylase activity serves as an indicator of the treatments' impact on the larvae's capacity to digest carbohydrates for energy. The α-amylase activity in the control group was 0.008 ± 0.0001 µg/min/mg protein. This indicates that the larvae that received no treatment metabolized carbohydrates normally. The control media group that was treated with yeast extract peptone dextrose broth medium had a slight reduction in α-amylase activity (0.0062 ± 0.0002 µg/min/mg protein). This suggests that the treatment with yeast extract peptone dextrose broth medium might slightly impair carbohydrate digestion. Diazinon 60% EC treatment reduced α-amylase activity (0.0074 ± 0.0001 µg/min/mg protein), indicating that the chemical pesticide interferes with carbohydrate digestion in *S. frugiperda* (Fig. 4). The decrease in enzyme activity implies that the larvae's ability to successfully digest starch and other carbohydrates was impaired, potentially resulting in energy shortages that could harm growth and development. Both *Saccharomyces* strains significantly reduced α-amylase activity. Larvae treated with *S. cerevisiae* HA-NY4 exhibited a decrease in α-amylase activity (0.0046 ± 0.0001 µg/min/mg protein), while those treated with *S. uvrum* HA-NY3 showed the lowest α-amylase activity (0.0029 ± 0.0001 µg/min/mg protein) (Fig. 4). *Saccharomyces* treatments significantly reduced α-amylase activity, suggesting that these strains may disrupt the larvae's digestive tract, limiting their ability to obtain energy from ingested starch. This could be a key mechanism by which certain fungal strains harm larvae and limit their ability to thrive.

Invertase (INV, EC 3.2.1.26) is one of the important enzymes of carbohydrate metabolism, especially in sucrose hydrolysis to glucose and fructose. These monosaccharides also are necessary for diverse metabolic functions, and differential invertase activity can give insight on how treatments affect sugar utility and energy metabolism. The invertase activity in the control group was 0.0942 ± 0.0023 µg/min/mg protein, which means that healthy larvae were breaking down sucrose normally (Fig. 4b). The control media group had modestly lower invertase activity (0.0788 ± 0.0007 µg/min/mg protein), showing that the medium may impede enzyme activity. The treatment group for the Diazinon 60% EC was found to significantly increase invertase activity (0.1002 ± 0.0015 µg/min/mg protein), but in a less pronounced manner in comparison with that seen for α-amylase activity. *Saccharomyces* treatments had different effects on invertase activity. *S. cerevisiae* HA-NY4 also affects the invertase activity significantly (0.1059 ± 0.0033 µg/min/mg protein), revealing that the strain causes significant stress, which leads to the imbalance in the process of sucrose digestion. *S. uvrum* HA-NY3 greatly reduced invertase activity (0.0746 ± 0.0032 µg/min/mg protein), reflecting that this strain is able to significantly inhibit sucrose metabolism in the larvae (Fig. 4b).

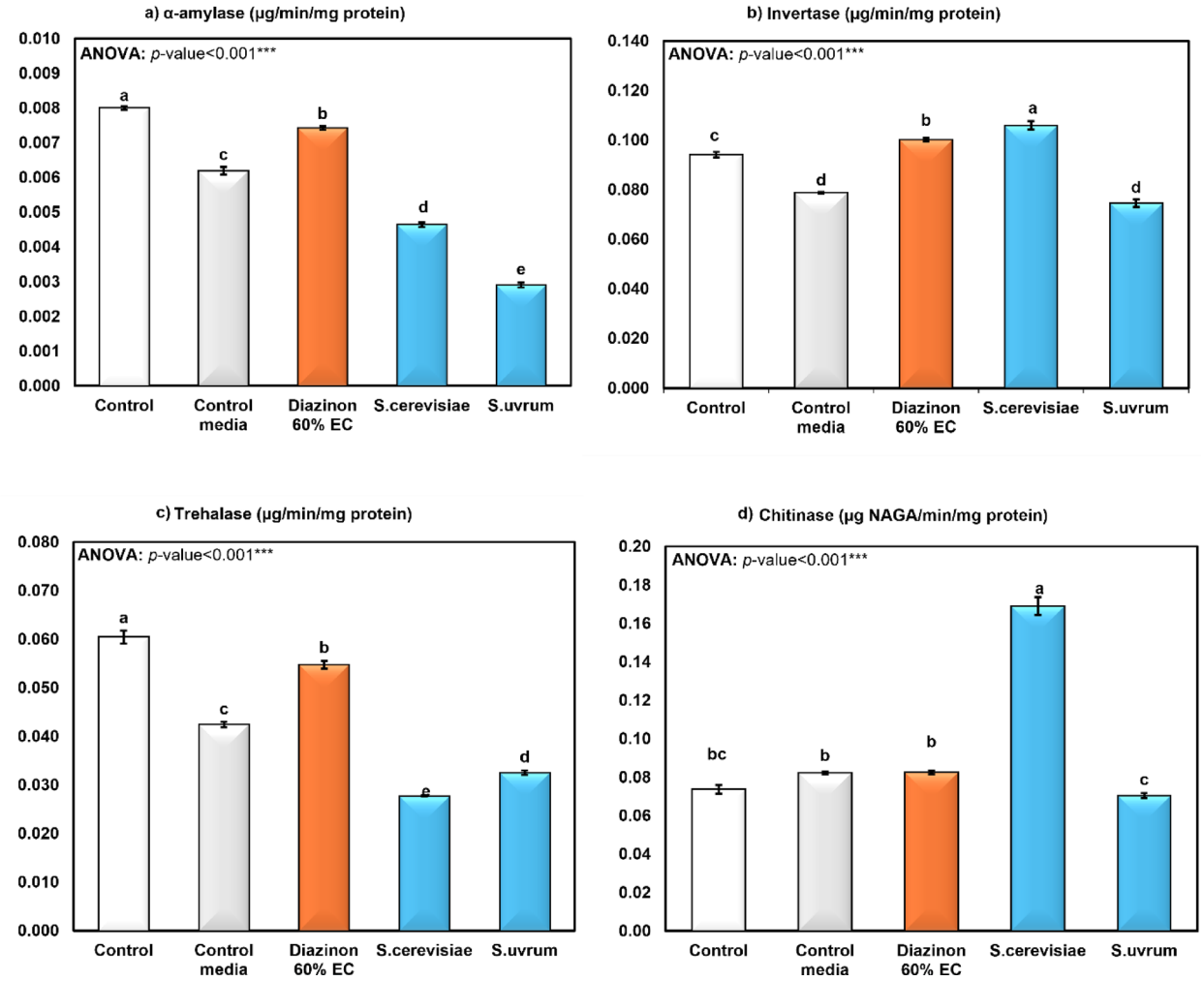

Fig. 4. Biochemical assessments of AMY, INV, TRE, and CHI of *S. frugiperda* larvae treated with *Saccharomyces* strains.

Trehalase (TRE, EC 3.2.1.28) is a significant hydrolytic enzyme in insects that catalyzes the hydrolysis of trehalose, a disaccharide acting as a prominent energy depot during stress and the developmental phases. Trehalose is the primary sugar in insect body weight and trehalase activity, an enzyme critical for the homeostatic regulation of energy balance. In untreated larvae, trehalose metabolism was considered normal as in the control group trehalase activity was (0.0604 ± 0.0026 µg/min/mg protein) (Fig. 4c). In the control media group, trehalase activity was decreased (0.0425 ± 0.0011 µg/min/mg protein), indicating that the yeast extract peptone dextrose broth medium has a certain blocking effect on this enzyme. Diazinon 60% EC treatment elevated trehalase activity (0.0547 ± 0.0016 µg/min/mg protein) when compared with the control media group (0.0425 ± 0.0011 µg/min/mg protein), suggesting that the chemical pesticide increases trehalose metabolism. However, *Saccharomyces* treatments suppressed trehalase activity to a greater extent, wherein *S. cerevisiae* HA-NY4 exhibited the lowest trehalase activity at (0.0277 ± 0.0002 µg/min/mg protein) and *S. uvrum* HA-NY3 showed slightly higher trehalase activity at (0.0325 ± 0.0009 µg/min/mg protein) (Fig. 4c). Both strains firmly disrupted trehalose metabolism, suggesting a possible defect in larvae's energy homeostasis and stress response. *Saccharomyces* strains significantly reduce trehalase activity. This indicates that these biocontrol agents influence the *S. frugiperda* metabolic pathways where it is important for survival and reproduction.

Chitinase (CHI, EC 3.2.1.14) hydrolyzes chitin, a significant component of insect cuticles, and is one of the most important of such enzymes. During molting and metamorphosis, chitinase activity is necessary for the degradation of the old exoskeleton and for the growth of the insect. This is one of the few studies demonstrating that larvae can normally degrade chitin, since in our control group chitinase activity reached 0.0737 ± 0.0046 µg NAGA/min/mg protein. The control media group had a slight increase in chitinase activity (0.0823 ± 0.0015 µg NAGA/min/mg protein), which suggests that the yeast extract peptone dextrose broth medium may stimulate chitinase production to some extent. The chemical pesticide Diazinon 60% EC had a non-significant effect on chitinase activity (0.0825 ± 0.0021 µg NAGA/min/mg protein), which means it didn't interfere with chitin metabolism in a significant way (Fig. 4d). *Saccharomyces* treatments greatly altered chitinase activity. The

chitinase activity of *S. cerevisiae* HA-NY4 is much higher (0.1690 ± 0.0092 µg NAGA/min/mg protein), which means that this strain stimulates the degradation of chitin in the larvae. Increased chitinase activity could result in early shedding of the exoskeleton or improper processing of exoskeleton formation that leads to deformities and mortality. The chitinase activity of *S. uvrum* HA-NY3 was slightly lower (0.0705 ± 0.0024 µg NAGA/min/mg protein), indicating that this strain could inhibit chitin metabolism and postpone molting and development (Fig. 4d). The feast-enzymatic activity in (Fig. 4d) indicates that *Saccharomyces* strains and Diazinon 60% EC made remarkable effects on those biochemical changes in *S. frugiperda* larvae.

Bars followed by different letters are significantly different according to Tukey's HSD at the 0.05 level.

AMY, represents α-amylase enzyme (AMY, EC 3.2.1.1) assessment; CHI, represents chitinase enzyme (CHI, EC 3.2.1.14) assessment; INV, represents invertase enzyme (INV, EC 3.2.1.26) assessment; TRE, represent trehalase enzyme (TRE, EC 3.2.1.28) assessment. Control, control *S. frugiperda* larval group treated with distilled water for experiment adjustment; Control media, control *S. frugiperda* larval group treated with yeast extract peptone dextrose broth medium for experiment adjustment; Diazinon 60% EC, *S. frugiperda* larval group treated with commercial pesticide Diazinon 60% EC as a positive control; *S. cerevisiae*, *S. frugiperda* larval group treated with *S. cerevisiae* HA-NY4 strain; *S. uvrum*, *S. frugiperda* larval group treated with *S. uvrum* HA-NY4 strain. We used Diazinon 60% EC at its recommended rate of 1 mL/L. All biochemical measurements were performed in triplicate.

Figure 5 presents the Pearson's correlation coefficient and two-tailed significance tests between study variables in a blue and red heatmap. Blue indicates positive correlation, red for negative correlation, and white for no correlation, with boxed blue or red for significant correlation. In addition, (Fig. 6) represents a canonical correspondence analysis presenting the interaction between study variables, where independent variables are presented by green arrows and dependent variables are presented by blue dots; the selected two CCA axes (CCA-1 and CCA-2) represent 95.6% of the total study variances. Both CCA axes (CCA-1 and CCA-2) exhibited highly significant variation among variables ($p < 0.0001$) with eigenvalues of CCA-1 = 0.038 and CCA-2 = 0.012. The negative inverse correlation of diazinon treatment with new adults, chitinase, and toxicity after 48 h was displayed using heat map and canonical correlation analysis (CCA) coordination. Larval longevity,

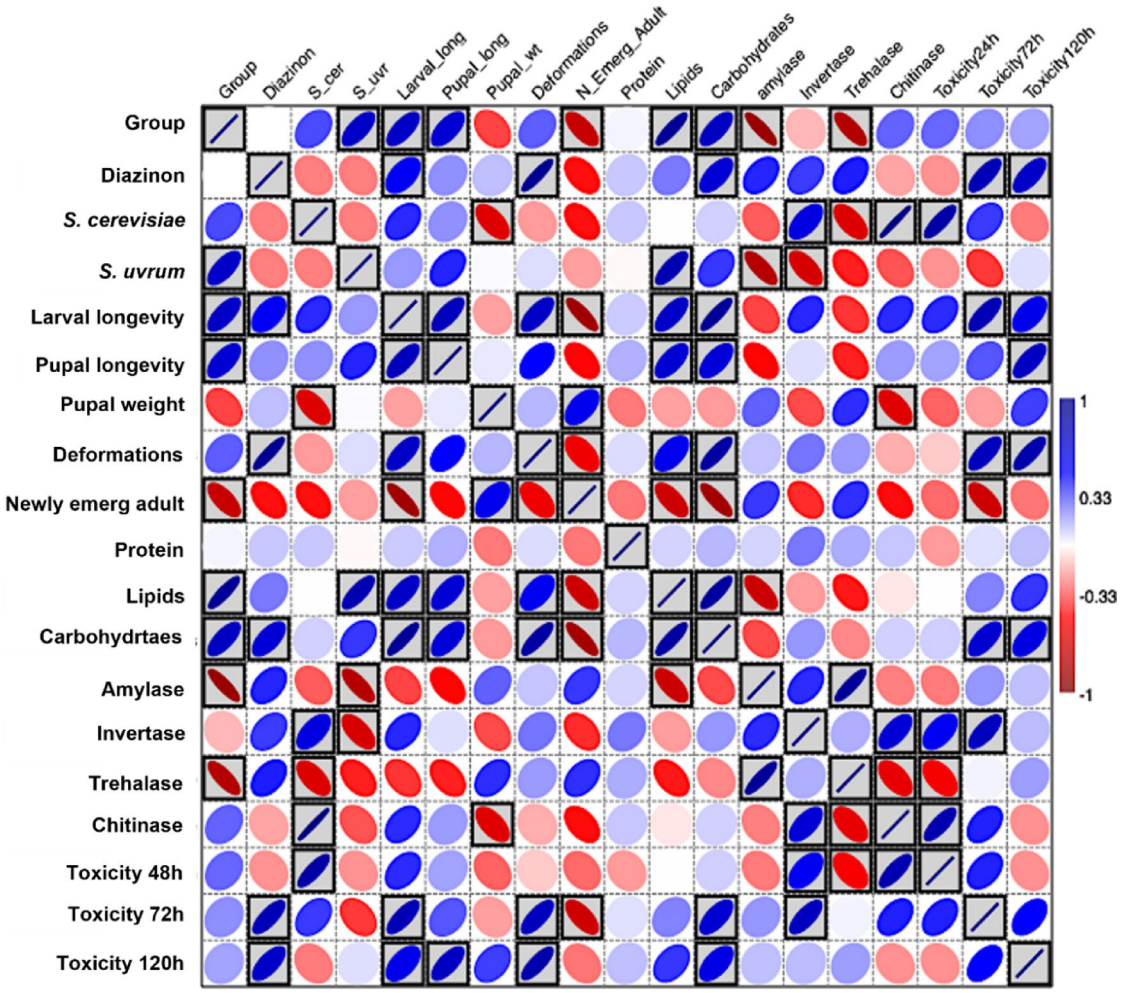

Fig. 5. Blue/Red heatmap presenting the Pearson's correlation coefficient and two-tailed significance tests between study variables.

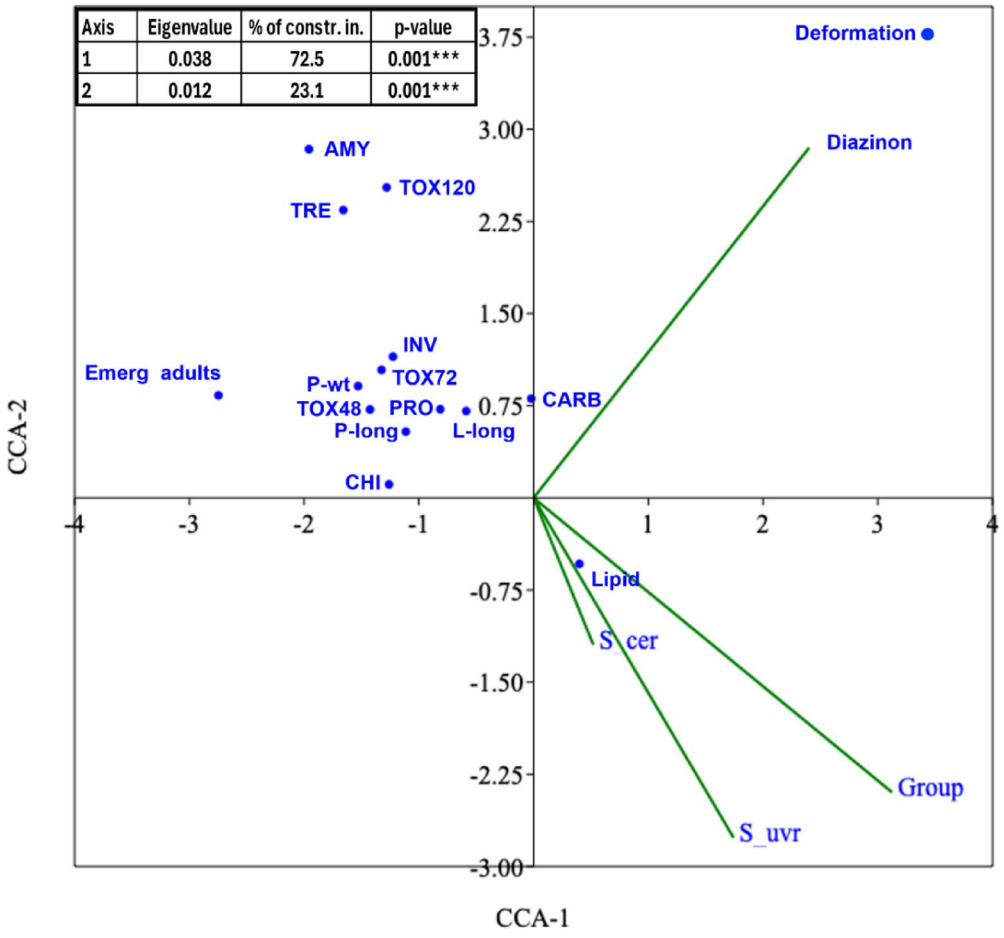

Fig. 6. Canonical Correspondence Analysis (CCA) ordination presenting the interaction between study variables.

pupal longevity, invertase, and chitinase had a positive correlation with *S. cerevisiae* HA-NY4. Furthermore, *S. uvrum* HA-NY3 positively correlates with most of the tested parameters, including larval longevity, pupal longevity, lipids, and carbohydrates, while it shows a negative correlation with α-amylase, invertase, trehalase, and chitinase activities and at toxicity at 48 and 72 h.

Blue indicates a positive correlation, red indicates a negative correlation, white indicates no correlation, and boxed blue or red indicates a significant correlation.

AMY, represents the α-amylase enzyme (AMY, EC 3.2.1.1); Carb, represents total carbohydrates; CHI, represents the chitinase enzyme (CHI, EC 3.2.1.14); Deform., represents deformations; Diaz., represents the *S. frugiperda* larval group treated with commercial pesticide Diazinon 60% EC; Emer-Ad., represents newly emerged *S. frugiperda* adults; INV, represents the invertase enzyme (INV, EC 3.2.1.26); Lipids, represent total lipids; L-long, represents larval longevity; Protein, represents total soluble protein; P-long, represents pupal longevity; P-wt, represents pupal weight; S_cer, represents the *S. frugiperda* larval group treated with *S. cerevisiae* HA-NY4; S_uvr, represents the *S. frugiperda* larval group treated with *S. uvrum* HA-NY3; TRE, represents trehalase enzyme (TRE, EC 3.2.1.28); TOX48, represents mortality rates 48 h post-treatment; TOX72, represents mortality rates 72 h post-treatment; TOX120, represents mortality rates 120 h post-treatment.

Green arrows represent the independent variables (factors), and blue dots indicate dependent variables.

AMY, represents the α-amylase enzyme (AMY, EC 3.2.1.1); CARB, represents total carbohydrates; CHI, represents the chitinase enzyme (CHI, EC 3.2.1.14); Diazinon, represents the *S. frugiperda* larval group treated with commercial pesticide Diazinon 60% EC; Emerg adults, represent newly emerged *S. frugiperda* adults; INV, represents the invertase enzyme (INV, EC 3.2.1.26); Lipid, represents total lipids; L-long, represents larval longevity; PRO, represents total soluble protein; P-long, represents pupal longevity; P-wt, represents pupal weight; S_cer, represents the *S. frugiperda* larval group treated with *S. cerevisiae* HA-NY4; S_uvr, represents the *S. frugiperda* larval group treated with *S. uvrum* HA-NY3; TRE, represents the trehalase enzyme (TRE, EC 3.2.1.28); TOX48, represents mortality rates 48 h post-treatment; TOX72, represents mortality rates 72 h post-treatment; TOX120, represents mortality rates 120 h post-treatment.

Discussion

Our main goal was to deeply study two *Saccharomyces* strains, *S. cerevisiae* HA-NY4 and *S. uvrum* HA-NY3, as entomopathogenic biocontrol agents against *S. frugiperda* larvae. Mortality, biological, and biochemical evaluations reveal that these *Saccharomyces* strains have promising outcomes for use in sustainable pest management systems. The toxicity rate of *Saccharomyces* strains at various time intervals is the first essential evidence of their performance as biocontrol agents. The results indicate that *S. cerevisiae* HA-NY4 significantly causes more mortality in *S. frugiperda* larvae than *S. uvrum* HA-NY3, particularly 72 h post-treatment. At 120 h, *S. cerevisiae* HA-NY4 demonstrated a high mortality rate, but *S. uvrum* HA-NY3 had fewer delays and severe toxic effects. *S. cerevisiae* HA-NY4 exhibits delayed mortality, indicating a gradual but effective mode of action. Our findings are nearly in line with Kelly et al.[24], who found a broad-acting mechanism combining epithelial damage and toxification in mosquitoes treated with *Saccharomyces cerevisiae*. While this mode of action may not produce immediate larvicidal effects, like those of chemical pesticides like Diazinon 60% EC, it does achieve significant mortality over time. This sequential toxicity is consistent with the action of a natural biocontrol agent, characterized typically by infection, metabolic alteration, and subsequent death of the larvae, as mentioned by Zayed et al.[25]. This mortality also exemplifies the long-term impacts of treated species, which substantially benefit from the use of *Saccharomyces* as an ecologically balanced control agent that minimizes the risks of non-target effects that are commonly observed by chemical insecticides[26]. Moreover, the low toxicity observed in the larvae at the early stages of treatment points out that *S. cerevisiae* HA-NY4 has lower immediate environmental impacts, and there is less probability of affecting beneficial insects and ecosystem predators[27].

In addition to direct toxicity, the biological aspects of *S. frugiperda* larvae provide us with a deeper understanding of how *Saccharomyces* treatments significantly affect prolonged larval and pupal longevity compared to the controls, as recommended by He et al.[28] in their work. This extended developmental period, particularly in the *S. cerevisiae* HA-NY4-treated larvae, suggests that the *Saccharomyces* treatments cause sublethal stresses on the larvae, delaying their progress toward maturity. This prolonged effect may be due to the sublethal stress or physiological disturbance caused by the treatments. Delays can considerably reduce the overall population growth rate of *S. frugiperda*, as larvae that take longer to develop are more susceptible to predation and environmental risks[29].

The rapid spread and effects of *S. frugiperda* on maize and other staple crops can be attributed to its high fecundity, broad host range, and strong migration ability. These characteristics render the fall armyworm a complex target to manage on a long-term basis, especially for smallholder farming where resources are constraints for adopting integrated pest management[12,30]. The decreased weight of pupae suggests that *S. cerevisiae* HA-NY4-treated larvae have nutritional deficits or metabolic disruptions that decrease their ability to amass sufficient materials to successfully pupate and emerge as adults. The observed decrease in pupate fitness may result in weaker adults with lower reproductive capability, contributing to limiting this pest population from reproducing, as suggested by Zayed et al.[25]. The deformities are consistent with this hypothesis, in which *S. cerevisiae* HA-NY4 appears to cause physiological stress, which then leads to developmental defects. This goes a long way in decreasing the number of reproductive adults[31]. The drastic decrease in the number of adults emerging from the *S. cerevisiae* HA-NY4-treated group establishes the potency of the fungus as a biocontrol agent. *S. cerevisiae* HA-NY4 maintains control over the *S. frugiperda* population in time by hindering the larvae from completing their life cycle[32]. As a result, these latent effects lower the chance of successful emergence as adults and further reproduction, thus enhancing the overall effectiveness of the treatments on pest suppression. Since the working mechanism for this is completely natural, compared to synthetic agrochemicals that frequently give rise to residues, this is effective and eco-friendly[33].

The biochemical assessments of *S. frugiperda* that were treated with different strains of *Saccharomyces* show how these biocontrol agents affect the metabolic processes of the pest[34]. *S. cerevisiae* HA-NY4 and *S. uvrum* HA-NY3 have a significant effect on the levels of total soluble proteins, lipids, and carbohydrates in the larvae, demonstrating that both treatments interfere with the larvae's ability to successfully metabolize nutrients. Insects use proteins for many of their basic developmental, metabolic, and immune mechanisms[35]. A decline in the total soluble proteins suggests that the *Saccharomyces* strains either limit the synthesis of this key molecule or enhance its degradation in the host, thereby compromising the fitness and growth of the larvae. Such biochemical dysregulation mirrors the reduced pupal weights and delays in development and connects metabolic maladaptation to a broad decline in the health and reproductive capacity of larvae[36]. Lipids are an important energy reserve and are needed to sustain cellular structure and function. Additionally, carbohydrates are a crucial source of energy for insects, allowing them to grow, develop, and survive[37]. The increase in total lipid and carbohydrate levels in the treated larvae suggests that *Saccharomyces* strains interfere with the larvae's energy metabolism, perhaps leading to an accumulation of reserves that the larvae are unable to effectively utilize[25]. This metabolic imbalance may explain the prolonged developmental periods observed[38]. The biochemical changes caused by *S. cerevisiae* HA-NY4 demonstrate that this strain may disturb a variety of metabolic pathways, making it an effective biocontrol agent.

The treatments with *Saccharomyces* strains had a significant impact on the activity of key enzymes for digestion (α-amylase, invertase, and trehalase) and the structural enzyme (chitinase)[39,40]. The reduction in α-amylase and invertase activities in the *S. cerevisiae* HA-NY4-treated group suggests that this strain may inhibit carbohydrate digestion, depriving larvae of vital energy sources for growth and molting[31]. The low trehalase activity suggests that the larvae cannot metabolize trehalose, a major insect metabolic sugar, which leads to energy deficits during important stages of development[41]. Inhibition of α-amylase, invertase, and trehalase activities indicates that *Saccharomyces* treatments influence the larvae's carbohydrate metabolism, which might cause energy deficits and thus growth reduction. On the other hand, the observation of increased chitinase activity in larvae treated with *S. cerevisiae* HA-NY4 implies that this strain facilitates the breakdown of chitin, the primary constituent of the insect exoskeleton, which agrees with the findings declared by Gotti et al.[32]. The

increase in chitinase activity may lead to structural weaknesses in the larvae's exoskeleton, resulting in molting failures or deformities[39]. The combination of metabolic and structural disruptions highlights the multifaceted nature of *S. cerevisiae* HA-NY4's biocontrol mechanisms, making it a potent agent for reducing the survival and reproductive capacity of *S. frugiperda*.

Saccharomyces treatments have the advantage of being more environmentally friendly than chemical pesticides[42]. Unlike Diazinon 60% EC, which poses risks to non-target organisms and leaves harmful environmental residues, *S. cerevisiae* HA-NY4 and *S. uvrum* HA-NY3 are naturally occurring yeast strains that do not produce toxic byproducts.

These yeast strains alter the biological processes of the insect not only by decreasing the activity of digestive enzymes but also by disrupting protein metabolism and inducing deformities in developing individuals[43].. Thus, the ability of *Saccharomyces* strains to be environmentally friendly provides a sustainable alternative to chemical pesticides, reducing the likelihood of pesticide resistance, water source contamination, and damage to beneficial agents such as pollinators and natural predators[44]. Importantly, the progressive and cumulative effects of *S. cerevisiae* HA-NY4 on *S. frugiperda* larvae, as evidenced by delayed but extensive mortality and developmental disruption, would further match the aim of integrative pest management (IPM)[45]. For this reason, IPM is advocated as the most environmentally sustainable, long-term solution that focuses on controlling pest populations whilst minimizing dependence on chemicals[46]. *S. cerevisiae* HA-NY4 has great potential to be incorporated into the general IPM strategies as it is effective against the isolation of pathogens and has a low environmental impact. This offers growers an effective, sustainable tool for *S. frugiperda* management. We recommended future research to test *Saccharomyces cerevisiae* HA-NY4 multiple doses for combating *S. frugiperda* and in-depth studying the modes of action.

In conclusion, *Saccharomyces cerevisiae* HA-NY4 is a promising strain for stopping *Spodoptera frugiperda* larvae from surviving, growing, and reproducing. This strain causes significant larval mortality as well as developmental delays, deformations, and metabolic dysfunctions that collectively reduce the pest's ability to survive. The eco-friendly nature of *S. cerevisiae* HA-NY4, along with its unique mechanism of action, makes it a promising biocontrol agent for achieving long-term pest management. People are becoming increasingly concerned about the health and environmental impacts of chemical insecticides such as Diazinon 60% EC. The *S. cerevisiae* HA-NY4 provides a potential alternative to lower *S. frugiperda* populations without harming the environment.

Material and methods

All chemicals and solvents were purchased from Sigma-Aldrich. The commercial pesticide, Diazinon 60% EC (DAWANA, Egypt), contains the active ingredient (O, O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate), which is an organophosphate pesticide. It contains 60% w/v Diazinon T.C. in the form of an emulsifiable concentrate. We used the commercial insecticide Diazinon 60% EC as a positive control in all bioassay investigations using its recommended rate (1 mL/L).

Ethics declarations

This research does not involve human participants; however, it does include insect studies. The Agricultural Research Center, Plant Protection Research Institute, and Imam Muhammad Ibn Saud Islamic University's ethical committees reviewed all protocols and techniques used in these insect experiments and approved them. All studies utilizing tested insects will follow the guidelines of the Food and Agriculture Organization of the United Nations (FAO).

Saccharomyces inocula

We purchased two identified *Saccharomyces* strains, *Saccharomyces cerevisiae* HA-NY4 (accession number KX588255) and *Saccharomyces uvrum* HA-NY3 (accession number KX588256), from the microbiology laboratory, botany and microbiology department, faculty of science, Zagazig University, Zagazig, Egypt. We purchased the strains as spore suspensions in 20% v/v glycerol (ADWIC, Egypt) at a temperature of – 15 °C.

Entomopathogen culture preparation

The studied *Saccharomyces* strains were grown from 2 µL ($6–9 \times 10^6$ conidia/mL) of spore suspension into 250 mL shake flasks that had 100 mL yeast extract peptone dextrose broth (1% yeast, 2% peptone, and 2% dextrose) medium. The flasks were then incubated at 28 ± 2 °C for 12 days with continuous shaking at 150 rpm. Once the cultures had reached the desired growth stage, transfer the yeast culture to sterile tubes and store it at 4 °C for short-term use[47].

Culture of *Spodoptera frugiperda* (J. E. Smith), Rearing technique

Egg masses of the *S. frugiperda* were collected from an open field in Abou Hammad city, Al-Sharqia Governorate, Egypt (30°28′24.6″N 31°39′01.5″E). This pest strain was grown for more than 18 generations without chemical contamination under controlled laboratory settings at 28 ± 2 °C, 70 ± 5% relative humidity, and a 12:12-h light–dark photoperiod. We provided the larvae with castor bean oil, *Ricinus communis* L., leaves as a nutritional resource. We gathered fresh castor leaves daily, washed them, and introduced them to the larvae after drying. We placed filter papers at the bottom of the jars to eliminate excess moisture. We created a pupation site at the base of the rearing jars, sexed newly emerging adult moths, and placed them in pairs in clean jars. Paper strips were introduced as oviposition sites. Fourth instar larvae were submitted for bioassay tests[48].

Bioassay of *Saccharomyces* strains' treatments (infection protocol)

Fourth instar larvae of recently molted *S. frugiperda* were starved for 4 to 5 h. We collected fresh castor leaves using scissors, thoroughly washed them, and cut them into equal weights and equal-sized pieces of leaves. These were then inoculated with the studied *Saccharomyces* cultures at a concentration ($1–2 \times 10^9$ cfu/mL) by a leaf-dipping technique assay[49], along with controls (sterile H_2O and yeast extract peptone dextrose broth medium) and Diazinon 60% EC at a concentration of 1 mL/L. The larvae were fed for 48 h with treated castor oil leaf slices and then replaced with fresh, untreated slices. There are eight larvae in each treated larval group with a weight ranging from 450 to 540 mg. All bioassay experiments were performed in triplicate. We assessed mortality against fourth-instar *S. frugiperda* larvae under controlled laboratory conditions. We investigated the mortality effect against *S. frugiperda* fourth instar larvae. We documented the number killed per day, known as mortality rates, 48, 72, and 120 h post-treatment by comparing the survival rates of infected larvae to those of the control groups and adjusted them using Abbott's formula[50]. We employed probit analysis, utilizing the calculated mortality percentages in relation to the respective concentrations[51].

Biological aspects

We looked at the controls and treated *S. frugiperda* larvae daily to see how long they stayed in the larval and pupal stages, how much they weighed as pupae, if they deformed, and when they hatched into adults[52].

Biochemical assessments

We used a double-beam ultraviolet/visible spectrophotometer (Spectronic 1201, Milton Roy Co., U.S.A.) to measure and quantify the absorbance of colored chemicals in *S. frugiperda* larvae. We collected the larvae 72 h post-treatment and stored them at − 20 °C. We centrifuged the homogenate samples of treated and control *S. frugiperda* larvae at 8000 rpm for 15 min at 5 °C. We then transferred the enzyme extracts into sterilized screw-capped tubes. The biochemical study aimed to understand the effects of *Saccharomyces* cultures' entomopathogenicity on *S. frugiperda* larvae. All enzymatic activities were quantified in enzyme units (EU) per milligram of protein content. All biochemical measurements were taken in triplicate.

Total soluble protein

Bradford's technique assessed the total protein concentration[53]. We used bovine serum albumin (Stanbio Laboratory, Texas, U.S.A.) as the standard to convert it to mg/mL. We quantified the samples' absorbance at 595 nm using a microplate reader. We measured the total soluble protein by mg/g body weight.

Total lipids

The protocol for lipid extraction and analysis was essentially described by the scientist Sun[54]. We extracted lipids from the cell homogenate by adding 4 mL of chloroform/methanol (2:1 v/v) to a 1 mL portion. We quantified the samples' absorbance at 525 nm and measured the total lipids by mg lipids/g fresh body weight.

Total carbohydrates

The methodology for carbohydrate extraction and analysis was fundamentally based on the procedures outlined by Van Handel[55]. Carbohydrates were extracted from the cell homogenate by incorporating 20% (w/v) phenol and sulfuric acid (H_2SO_4) into the samples. We evaluated the absorbance of the samples at 490 nm and assessed the total carbohydrates in µg glucose/g fresh body weight.

α-amylase enzyme (AMY, EC 3.2.1.1) determination

The alpha-amylase enzyme (AMY) activity was measured using a starch digestion assay coupled with a colorimetric detection[56]. The absorbance of the samples was measured at 550 nm against a blank of soluble starch in phosphate buffer. We expressed the activity as µg/min/mg protein.

Invertase enzyme (INV, EC 3.2.1.26) determination

We measured the invertase enzyme (INV) activity by catalyzing the hydrolysis of sucrose into reducing sugars and using a colorimetric assay with the 3,5-dinitrosalicylic acid (DNS) (Sigma-Aldrich, Germany) reagent[57]. The absorbance of the samples was measured at 550 nm, comparing them to a blank solution of sucrose in phosphate buffer. The activity was expressed as µg/min/mg protein.

Trehalase enzyme (TRE, EC 3.2.1.28) determination

The trehalase enzyme (TRE) activity was measured based on catalyzing the hydrolysis of trehalose into glucose molecules, followed by a colorimetric detection[58]. The absorbance of the samples was measured at 550 nm against a blank solution of trehalose in phosphate buffer. The activity was expressed as µg/min/mg protein.

Chitinase enzyme (CHI, EC 3.2.1.14) determination

We used a modified spectrophotometric method[59] to measure the chitinase while keeping the pH at 6.5 with phosphate-acetate buffer. Cutting β-1,4 glycosidic linkages between nearby N-acetyl glucosamines in the chitin chain is what the method does. We measured the samples at a wavelength of 540 nm in comparison to a blank of N-acetyl glucosamine. The activity was measured as µg NAGA/min/mg protein.

Statistical data analysis

All *Saccharomyces* cultures and assays were performed in triplicate, and the findings were represented as dry weight. Data were checked for normality using the Shapiro–Wilk and Kolmogorov–Smirnov tests; accordingly, data were parametric. Accordingly, the data was presented as the mean value (n = 3) ± standard deviation (SD).

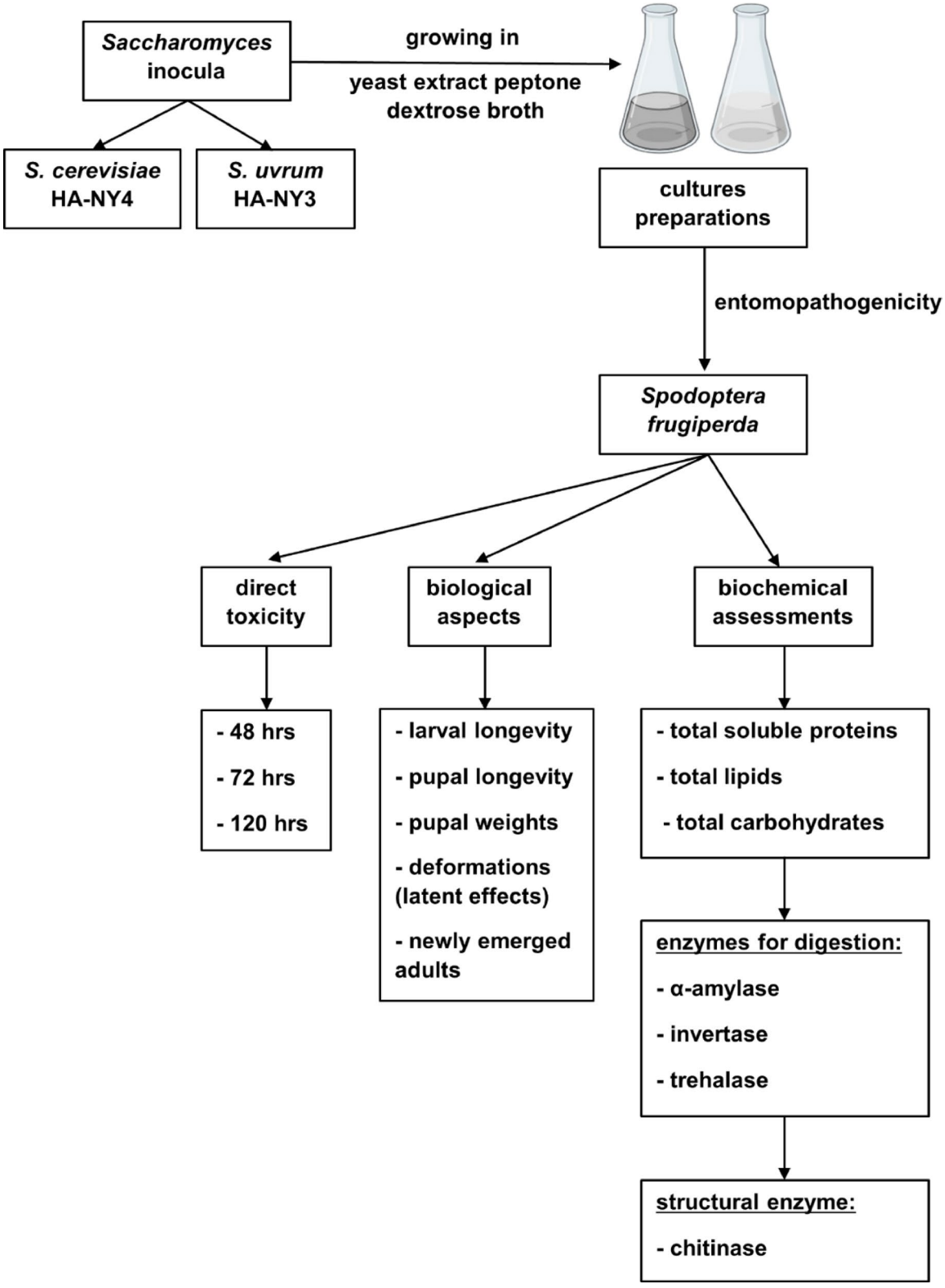

Fig. 7. Graphic flowchart demonstrating the research process of the current study.

Inferential statistics to check the difference between different treatment groups were evaluated using one-way analysis of variance followed by Tukey's HSD post hoc test at a 0.05 significance level. All statistical analyses were conducted using IBM-SPSS statistical software (version 30.0 of Mac OS, SPSS Inc., Chicago, IL, U.S.A.). Pearson's correlation coefficients between entomopathogenic treatments and biocidal efficacy were calculated

and presented in terms of a blue-red heatmap. Canonical correspondence analysis (CCA) was also performed to check the interaction between study variables. Heatmap and CCA were conducted using PAST statistical software version 4.04 for Mac OS. (Fig. 7) clarifies the strategy of this research process.

Data availability

The authors declare that the datasets presented in this study are available on request from the corresponding author.

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References

1. Abbas, A. et al. Biological control of fall armyworm. *Spodoptera frugiperda. Agronomy* **12**, 2704. https://doi.org/10.3390/agronomy12112704 (2022).
2. Mendesil, E. et al. The invasive fall armyworm, *Spodoptera frugiperda*, in Africa and Asia: Responding to the food security challenge, with priorities for integrated pest management research. *J. Plant Dis. Prot.* **130**, 1175–1206. https://doi.org/10.1007/s41348-023-00777-x (2023).
3. Montezano, D. G. et al. Host Plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *Afr. Entomol.* **26**, 286–300. https://doi.org/10.4001/003.026.0286 (2018).
4. Ayra-Pardo, C. & Borras-Hidalgo, O. Fall Armyworm (FAW; Lepidoptera: Noctuidae): Moth Oviposition and Crop Protection. in *Olfactory Concepts of Insect Control - Alternative to insecticides* (ed. Picimbon, J.-F.) 93–116 (Springer International Publishing, 2019). https://doi.org/10.1007/978-3-030-05060-3_5.
5. Kushwaha, U. K. S. A cost-efficient and alternative technique of managing fall armyworm Spodoptera frugiperda (J.E. Smith) larvae in maize crop. *Sci. Rep.* **12**, 6741. https://doi.org/10.1038/s41598-022-10982-7 (2022).
6. Dos Santos, M. V. C. et al. Performance of fall armyworm preimaginal development on cultivars of tropical grass forages. *Insects* **13**, 1139. https://doi.org/10.3390/insects13121139 (2022).
7. Chen, Y. et al. P450 gene *CYP321A8* is responsible for cross-resistance of insecticides in field populations of *Spodoptera frugiperda*. *Insect Sci.* **32**, 227–242. https://doi.org/10.1111/1744-7917.13376 (2025).
8. Chimweta, M., Nyakudya, I. W., Jimu, L. & Bray Mashingaidze, A. Fall armyworm [Spodoptera frugiperda (JE Smith)] damage in maize: Management options for flood-recession cropping smallholder farmers. *Int. J. Pest Manag.* **66**, 142–154. https://doi.org/10.1080/09670874.2019.1577514 (2020).
9. Fenibo, E. O., Ijoma, G. N. & Matambo, T. Biopesticides in sustainable agriculture: Current status and future prospects. in *New and Future Development in Biopesticide Research: Biotechnological Exploration* (eds. Mandal, S. D., Ramkumar, G., Karthi, S. & Jin, F.) 1–53 (Springer Nature Singapore, 2022). https://doi.org/10.1007/978-981-16-3989-0_1.
10. Chen, J. et al. The dynamics of the microbial community in fall armyworm *Spodoptera frugiperda* during a life cycle. *Entomol. Exp. Appl.* **171**, 502–513. https://doi.org/10.1111/eea.13282 (2023).
11. Gohel, N. M., Raghunandan, B. L., Patel, N. B., Parmar, H. V. & Raval, D. B. Role of fungal biocontrol agents for sustainable agriculture. In *Fungal Diversity, Ecology and Control Management* (eds. Rajpal, V. R., Singh, I. & Navi, S. S.) 577–606 (Springer Nature Singapore, 2022). https://doi.org/10.1007/978-981-16-8877-5_28.
12. Wyckhuys, K. A. G. et al. Global scientific progress and shortfalls in biological control of the fall armyworm *Spodoptera frugiperda*. *Biol. Control* **191**, 105460. https://doi.org/10.1016/j.biocontrol.2024.105460 (2024).
13. Liu, D., Smagghe, G. & Liu, T.-X. Interactions between Entomopathogenic Fungi and Insects and Prospects with Glycans. *J. Fungi* **9**, 575. https://doi.org/10.3390/jof9050575 (2023).
14. Irsad, et al. Entomopathogen-based biopesticides: Insights into unraveling their potential in insect pest management. *Front. Microbiol.* **14**, 1208237. https://doi.org/10.3389/fmicb.2023.1208237 (2023).
15. Kaur, M., Mohammad Said Al-Tawaha, A. R. & Karnwal, A. The beneficial impact of microbes in food production, health, and sustainability. In *Food Microbial Sustainability* (eds. Karnwal, A. & Mohammad Said Al-Tawaha, A. R.) 289–309 (Springer Nature Singapore, 2023). https://doi.org/10.1007/978-981-99-4784-3_14.
16. Jeong, W.-S., Kong, H.-R., Kim, S.-Y. & Yeo, S.-H. Exploring the Health Benefits of Yeast Isolated from Traditional Fermented Foods in Korea: Anti-Inflammatory and Functional Properties of *Saccharomyces* and Non-*Saccharomyces* Strains. *Microorganisms* **11**, 1503 https://doi.org/10.3390/microorganisms11061503 (2023).
17. Singh, V. Microbial bioactive components: Sources, applications, and sustainability. In *Bioactive Components* (eds. Thakur, M. & Belwal, T.) 103–117 (Springer Nature Singapore, 2023). https://doi.org/10.1007/978-981-19-2366-1_7.
18. Parapouli, M. et al. *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol.* **6**, 1–32. https://doi.org/10.3934/microbiol.2020001 (2020).
19. Abid, R. et al. Probiotic Yeast *Saccharomyces*: Back to Nature to Improve Human Health. *J. Fungi* **8**, 444. https://doi.org/10.3390/jof8050444 (2022).
20. Yang, H. et al. Boosting production of cembratriene-ol in *Saccharomyces cerevisiae* via systematic optimization. *Biotechnol. J.* **19**, 2300324. https://doi.org/10.1002/biot.202300324 (2024).
21. Zveushe, O. K. et al. Effects of Co-Inoculating Saccharomyces spp. with Bradyrhizobium japonicum on Atmospheric Nitrogen Fixation in Soybeans (Glycine max (L.)). *Plants* **12**, 681. https://doi.org/10.3390/plants12030681 (2023).
22. Srikamwang, C. et al. Role of microbial volatile organic compounds in promoting plant growth and disease resistance in horticultural production. *Plant Signal. Behav.* **18**, 2227440. https://doi.org/10.1080/15592324.2023.2227440 (2023).
23. Riseh, R. S., Vazvani, M. G. & Kennedy, J. F. β–glucan-induced disease resistance in plants: A review. *Int. J. Biol. Macromol.* **253**, 127043. https://doi.org/10.1016/j.ijbiomac.2023.127043 (2023).
24. Kelly, P. H., Yingling, A. V., Ahmed, A., Hurwitz, I. & Ramalho-Ortigao, M. Defining the mechanisms of action and mosquito larva midgut response to a yeast-encapsulated orange oil larvicide. *Parasit. Vectors* **15**, 183. https://doi.org/10.1186/s13071-022-05307-6 (2022).
25. Zayed, M. S. et al. Influence of Effective Microorganisms on Some Biological and Biochemical Aspects of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Life* **12**, 1726. https://doi.org/10.3390/life12111726 (2022).
26. Giorio, C. et al. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 1: new molecules, metabolism, fate, and transport. *Environ. Sci. Pollut. Res.* **28**, 11716–11748. https://doi.org/10.1007/s11356-017-0394-3 (2021).
27. Malassigné, S. et al. Diversity and functions of yeast communities associated with insects. *Microorganisms* **9**, 1552. https://doi.org/10.3390/microorganisms9081552 (2021).
28. He, H. et al. The frequency of cannibalism by *Spodoptera frugiperda* larvae determines their probability of surviving food deprivation. *J. Pest Sci.* **95**, 145–157. https://doi.org/10.1007/s10340-021-01371-6 (2022).
29. Holkenbrink, C. et al. Production of moth sex pheromones for pest control by yeast fermentation. *Metab. Eng.* **62**, 312–321. https://doi.org/10.1016/j.ymben.2020.10.001 (2020).

30. Sousa, F. F. et al. Life-history traits of *spodoptera frugiperda* populations exposed to low-dose Bt Maize. *PLoS ONE* **11**, e0156608. https://doi.org/10.1371/journal.pone.0156608 (2016).
31. Wang, B., Huang, D., Cao, C. & Gong, Y. Insect α-amylases and their application in pest management. *Molecules* **28**, 7888. https://doi.org/10.3390/molecules28237888 (2023).
32. Gotti, I. A., Moreira, C. C., Delalibera, I. & De Fine Licht, H. H. Blastospores from *Metarhizium anisopliae* and *Metarhizium rileyi* are not always as virulent as conidia are towards *Spodoptera frugiperda* caterpillars and use different infection mechanisms. *Microorganisms* **11**, 1594. https://doi.org/10.3390/microorganisms11061594 (2023).
33. Liess, M. & Gröning, J. Latent pesticide effects and their mechanisms. *Sci. Total Environ.* **909**, 168368. https://doi.org/10.1016/j.scitotenv.2023.168368 (2024).
34. Yang, X. et al. Pathogenicity, infection process, physiological and biochemical effects of Metarhizium rileyi against Spodoptera frugiperda (J.E. Smith) (Lepidoptera: Noctuidae) larvae. *Egypt. J. Biol. Pest Control* **34**, 19. https://doi.org/10.1186/s41938-024-00781-9 (2024).
35. Wojda, I., Cytryńska, M., Zdybicka-Barabas, A. & Kordaczuk, J. Insect defense proteins and peptides. In *Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and other Body Fluid Proteins* (eds. Hoeger, U. & Harris, J. R.) vol. 94 81–121 (Springer International Publishing, 2020).
36. Shu, B. et al. Growth inhibition of *Spodoptera frugiperda* larvae by camptothecin correlates with alteration of the structures and gene expression profiles of the midgut. *BMC Genomics* **22**, 391. https://doi.org/10.1186/s12864-021-07726-8 (2021).
37. Wrońska, A. K., Kaczmarek, A., Boguś, M. I. & Kuna, A. Lipids as a key element of insect defense systems. *Front. Genet.* **14**, 1183659. https://doi.org/10.3389/fgene.2023.1183659 (2023).
38. Salem, S. A. R., Alhousini, E. M. E., Al-Amgad, Z. & Mahmoud, M. A. B. Efficiency of spinetoram on biological, biochemical, and histological parameters in the invasive fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Egypt. *J. Plant Dis. Prot.* **131**, 489–499. https://doi.org/10.1007/s41348-023-00835-4 (2024).
39. Barbole, R. S., Sharma, S., Patil, Y., Giri, A. P. & Joshi, R. S. Chitinase inhibition induces transcriptional dysregulation altering ecdysteroid-mediated control of *Spodoptera frugiperda* development. *iScience* **27**, 109280. https://doi.org/10.1016/j.isci.2024.109280 (2024).
40. Dos Santos Moreira, L. M. et al. Assessment of the entomopathogenic potential of fungal and bacterial isolates from fall armyworm cadavers against *Spodoptera frugiperda* Caterpillars and the Adult Boll Weevil, Anthonomus grandis. *Neotrop. Entomol.* **53**, 889–906. https://doi.org/10.1007/s13744-024-01159-0 (2024).
41. Tang, B. et al. Regulation of three novel pepper thiothiazolidinones on the fecundity of *Spodoptera frugiperda*. *Pestic. Biochem. Physiol.* **204**, 106033. https://doi.org/10.1016/j.pestbp.2024.106033 (2024).
42. Villena, G. K., Ludeña, Y. & Samolski, I. Applications of yeast for environmental clean-up and sustainable agriculture. In *Advances in Yeast Biotechnology for Biofuels and Sustainability* 193–218 (Elsevier, 2023). https://doi.org/10.1016/B978-0-323-95449-5.00018-7.
43. Fu, J. et al. Composition and diversity of gut microbiota across developmental stages of *Spodoptera frugiperda* and its effect on the reproduction. *Front. Microbiol.* **14**, 1237684. https://doi.org/10.3389/fmicb.2023.1237684 (2023).
44. Furlan, L. et al. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 3: Alternatives to systemic insecticides. *Environ. Sci. Pollut. Res.* **28**, 11798–11820. https://doi.org/10.1007/s11356-017-1052-5 (2021).
45. Vermelho, A. B., Moreira, J. V., Akamine, I. T., Cardoso, V. S. & Mansoldo, F. R. P. Agricultural pest management: The role of microorganisms in biopesticides and soil bioremediation. *Plants* **13**, 2762. https://doi.org/10.3390/plants13192762 (2024).
46. Zhou, W. et al. Integrated pest management: An update on the sustainability approach to crop protection. *ACS Omega* **9**, 41130–41147. https://doi.org/10.1021/acsomega.4c06628 (2024).
47. De Llanos, R., Fernández-Espinar, M. T. & Querol, A. A comparison of clinical and food *Saccharomyces cerevisiae* isolates on the basis of potential virulence factors. *Antonie Van Leeuwenhoek* **90**, 221–231. https://doi.org/10.1007/s10482-006-9077-7 (2006).
48. Khedr, M. M. A., Amer, A. E. A., Nada, M. M. & El-Sayed, A. A. A. Preliminary observations and domestic management of the polyphagous fall armyworm, (*Spodoptera Frugiperda*) in Sharqia Governorate. *Egypt. J. Agric. Sci. Sri Lanka* **19**, 530–547. https://doi.org/10.4038/jas.v19i3.10372 (2024).
49. Khedr, M. & El-Kawas, H. Control of Spodoptera littoralis (Boisd.) (Lepidoptera:Noctuidae) and Tetranychus urticae Koch (Acari:Tetranychidae) by Coriander Essential Oil. *J. Entomol.* **10**, 170–181. https://doi.org/10.3923/je.2013.170.181 (2013).
50. Abbott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18**, 265–267. https://doi.org/10.1093/jee/18.2.265a (1925).
51. Wiegand, H. Finney, D. J. Probit analysis. 3. Aufl. Cambridge University Press, Cambridge 1971. XV, 333 S., 41 Rechenbeispiele, 20 Diagr., 8 Tab., 231 Lit., L 5.80. *Biom. Z.* **14**, 72–72. https://doi.org/10.1002/bimj.19720140111 (1972).
52. Farrar, R. R., Barbour, J. D. & Kennedy, G. G. Quantifying food consumption and growth in insects. *Ann. Entomol. Soc. Am.* **82**, 593–598. https://doi.org/10.1093/aesa/82.5.593(1989).
53. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3(1976).
54. Sun, G. Y. Preparation and analysis of acyl and alkenyl groups of glycerophospholipids from brain subcellular membranes. In *Lipids and Related Compounds* vol. 7 63–82 (Humana Press, 1989).
55. Van Handel, E. Rapid determination of glycogen and sugars in mosquitoes. *J. Am. Mosq. Control Assoc.* **1**, 299–301 (1985).
56. Gutierrez, C. et al. α-amylase activities of agricultural insect pests are specifically affected by different inhibitor preparations from wheat and barley endosperms. *Plant Sci.* **72**, 37–44. https://doi.org/10.1016/0168-9452(90)90184-P (1990).
57. Ishaaya, I. & Swirski, E. Trehalase, invertase, and amylase activities in the black scale, Saissetia oleae, and their relation to host adaptability. *J. Insect Physiol.* **22**, 1025–1029. https://doi.org/10.1016/0022-1910(76)90087-1 (1976).
58. Dahlqvist, A. Assay of intestinal disaccharidases. *Anal. Biochem.* **22**, 99–107. https://doi.org/10.1016/0003-2697(68)90263-7 (1968).
59. Bolognesi, R. et al. Sequences of cDNAs and expression of genes encoding chitin synthase and chitinase in the midgut of *Spodoptera frugiperda*. *Insect Biochem. Mol. Biol.* **35**, 1249–1259. https://doi.org/10.1016/j.ibmb.2005.06.006 (2005).

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Author contributions

All authors contributed to the study. M.D., A.A., E.G., E.A., A.E. and M.S. conceived the conceptualization and designed research. M.D., E.G., E.A. and M.S. conducted investigation, methodology, resources, data curation, and validation. A.A., E.G. and A.E. contributed formal analysis and visualization. M.D., E.G. and E.A. wrote the original draft of the manuscript, and all authors reviewed and commented on previous versions of the manuscript. A.A. implemented funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests
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Consent to participate
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