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Inducing the resistance against *Tobacco mosaic virus* in pepper using the extracts of *Datura stramonium* and *Ganoderma lucidum*

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ABSTRACT

pepper plants treated with the same Tobacco mosaic virus (TMV) showed no signs and symptoms of disease, in contrast to plants treated with these compounds as a control, which was thought to act as an inhibitory start to the viral infection. This resistance was sparked by the pepper plants' exposure to the TMV virus. Furthermore, the ELISA test findings indicated a wave reaction based on the yellow color that developed in the ELISA plate wells containing the extracts of pepper plants that have been treated with *Ganoderma lucidum* extract. When compared to the control treatment, which is infected with the virus alone, the wave absorption amount at 405 nm was 0.296 nm, indicating that the highest percentage of virus duplication occurred (69%) after the second week of the *G. lucidum* treatment and before six days of infection. On the other hand, 58% of the virus was duplicated when the *G. lucidum* extract was sprayed three days prior to the virus infection, and the wave absorption value was 0.404 nm. The values of wave absorption following splashing with the same extract were 0.612 and 0.598 after three and six days of virus infection, respectively, and the percentages of virus duplication were 37 and 38%. Lastly, it should be emphasized that, rather than using chemical pesticides that are unsafe to use against the causes of plant diseases, more research must be done in the future regarding the extraction of these anti-viral plant extracts as well as extracts of naturally occurring substances that are environmentally friendly.

Keywords: Induced antiviral proteins (IAVPs), *Tobacco mosaic virus*, *Datura stramonium*, *Ganoderma lucidum*.

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INTRODUCTION

The main goal of those who specialize in plant diseases is thought to be controlling viruses and the diseases they generate. There is no direct means to fight viruses because of their nature, which includes their complete attachment to the infected plant's cells and their presence inside the cells without any separation from the components of the cells. Although chemicals were quite successful in treating a variety of bacterial and fungal infections, no appreciable progress was made against viral problems (Zaitlin and Hull, 1987; Makkouk *et al*., 2008). There are now just a few ways available to control plant viruses by controlling the insects that transmit them, despite the fact that several fungal, bacterial, nematodes, and other causes as well as other epidemics have been handled by making use of these strategies in disease management. Because of the uncontrollably high losses in agricultural crops on a global scale (Briddon and Markham, 2000; Scholthof *et al.*, 2011), plant viruses are considered one of the most significant issues facing agricultural production. It will take a while to find a solution for this issue. As a result, numerous initiatives were undertaken to combat the viruses; the majority of these approaches indirectly targeted the viruses in an effort to stop their spread and reduce the host's losses, but the results fell short of expectations (Lowe, 2014;

Wasser and Weis, 2022). Therefore, it has become necessary to find inexpensive, effective, and environmentally acceptable materials. One of the best ways to ensure sustainable agricultural production with the least amount of environmental damage is to use natural materials as pesticides. According to (Sivanandhan *et al.,* 2017) and Rogers (2006) and Newbury and Possingham (1977), a number of substances with an inhibitory effect were extracted from plants belonging to family the Amaranthaceae, Caryophyllaceae, Leguminosae, , and Solanaceae ,as well as from the fruit bodies of the Basidiomycetes fungus. These substances demonstrated an inhibitory effect on the growth of viruses as well as animal and plant fungus.

Four categories of anti-virus chemicals were identified by Chessin *et al.* (1995), furanocoumarin, alkaloid, terpenoid, and lignin. Some of these substances have been identified as protein-like, with weights ranging from 1000 to 38000 Dalton. They are broad-spectrum virus inhibitors, thermally stable, precipitable at high ammonium sulfate concentrations, and comparable to animal interferons with the exception of their performic acid sensitivity (Nadu, 2014). Owing to the broad application of the generated compounds against plant viruses, whether of animal or chemical origin, the investigator decided to carry out this investigation, which entails applying the potent compounds to the *Ganoderma lucidum* fungus and Datura stramonium plant and determining how they affect the Tobacco mosaic virus (Wasser and Weis,1999; Choudhary *et al.*, 2020).

MATERIALS AND METHODS

Repairing the bean plants-Tobacco mosaic virus

In the greenhouse at the Department of Plant Protection, College of Agriculture and Forestry at Mosul University, the bean plants were planted in order to retain the isolate of the Tobacco mosaic virus (TMV). This came about following the preparation of the 20-centimeter-diameter plastic pots. Previously, the soil was formalin-sterilized at a concentration of 4%, diluted with water, and combined with peat moss at a 2:1 ratio to boost soil fertility and encourage plant growth. The majority of the bean plant leaves were injected with the virus under investigation after germination; this virus has previously been identified by DAS-ELISA. The plants were housed in the greenhouse, where they received regular irrigation, NPK fertilization, and frequent monitoring until the disease symptoms were evident on them.

Antiviral Proteins Induced Purification from *Datura stramonium* plant leaves

Five grams of fresh Datura plant leaves were ground (crushed) in a ceramic mortar, and ten milliliters of a pH-7 buffer solution were added. This was done using the Zipf (1987) method. The mixture was passed through two mousseline layers, collecting the liquid free of impurities and placing it in a sterile baker. Subsequently, incorporate an equivalent volume of 70% saturated ammonium sulfate ($NH_4(So_4)_2$) into the extract. Allow the mixture to sit at room temperature for two hours, or until turbidity is noticed, a sign of protein precipitation. After centrifuging the turbid mixture for 30 minutes at 5,000 rpm, the precipitate was collected. The precipitate was dissolved in 0.5 ml of phosphate buffer solution, and a membrane separation was performed to eliminate the ammonium sulfate. This involved placing the precipitate suspension in a separation tube and then placing the tube inside a volume flask that held one liter of (PBS) buffer. For 30 minutes, the magnetic rotor was used to stir continuously during the membrane separation process. After adding 0.5 ml of PBS buffer, the contents of the resulting precipitate were centrifuged at a high speed for four minutes (13000 rpm). The precipitate was then dissolved in 0.5 ml of PBS buffer, and the plant proteins extracted from the *Datura stramonium* plant were estimated using the Givan and Leech (1971) method by measuring the photo absorptivity in spectrophotometer type and using the following equation:

Absorption at 280 nm – absorption at 253/2.51 equals protein concentration (g/ml)

The molecular weight of *Datura stramonium* internal proteins

Using the SDS-polyacrylamide electrophoresis (SDS-PAGE) method in a poly-acrylamide gel, the molecular weight of the effective proteins was estimated to be 15% in the separation gel. The contents were heated to 100°C in a 0.21 molar Tris-HCl buffer solution containing 0.05 of bromophenol blue dye, 15% glycerol, 2% SDS, and 2% -mercaptoethanol-2. Samples were placed on the gel surface, and electrophoresis was carried out by subjecting the gel to a 100 volt electrical potential for five hours. Subsequently, the gel was submerged in a solution containing 0.25% commasie blue stain, 5 volumes of methanol, 5 volumes of sterilized water, 1 volume of acetic acid, and 5% of methanol at 37°C, while being gently stirred to eliminate the dye (Garfin, 1990).

Performic acid treatment of proteins isolated from the Datura plant

After treating the Datura plant's proteins with 10 milliliters of performic acid, the mixture was placed in an electrical vibrator for 60 minutes, centrifuged for 30 minutes at 3000 rpm, and the precipitate was separated using the previously described poly-acrylamide gel electrophoresis technique.

Making the *Ganoderma lucidum* extract from Reishi mushrooms

The Reishi mushroom fungus extract was purchased as a powder from the Malaysian company DXN-SDN BMD-Pharmaceutical. In order to do the experiment, one gram of the powder was obtained and combined with ten milliliters of sterile water.

RESULTS AND DISCUSSION

Datura extracts induced antiviral proteins (IAVPs)

According to estimates of protein concentration, the healthy Datura plant has a concentration of 201.3 g/ml, whereas the TMV virus-injected plants had a concentration of 233.1 g/ml for the identical plants. This suggests that following the viral infection, the Datura plant produced novel proteins. By using the molecular indicators of the standard proteins—Rabbit muscle phosphorylase Bovine, Bovine serum albumin, Hen egg white ovalbumin, Bovine carbonic anhydrase, and Soybean trypsin inhibitor—two protein bands with weights of 31 and 80.1 kilo Daltons emerged from the extracts analysis in the poly-acrylamide gel. These proteins have molecular weights of 90500, 65000, 21500, 31000, and 46000, respectively, (Van Damme *et al.*, 2021). Only the same induced bands showed up when the proteins extracted from the Tobacco mosaic virus-infected Datura plants were combined with Performic acid and subjected to polyacrylamide gel electrophoresis (Figure 1), indicating that these are compounds that resulted from inducing resistance with plant interferon characteristics.

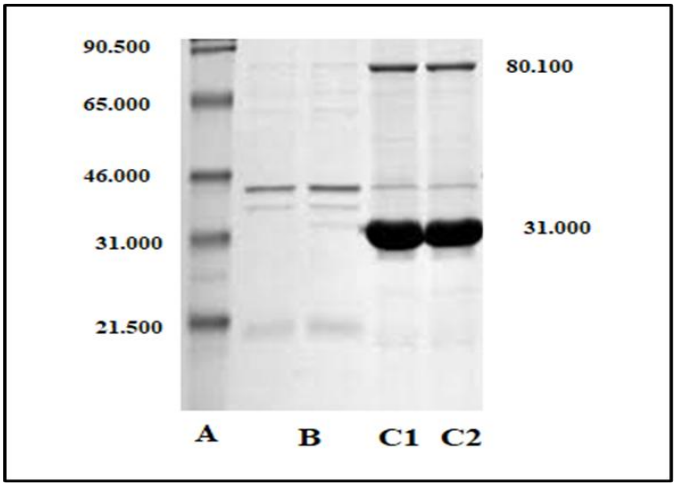

Figure 1. The electrophoresis of Datura protein extract in the polyacrylamide gel. A = standard proteins, B = proteins extracted from healthy Datura plants, C1 = proteins extracted from healthy Datura plants infected with TMV virus, C2 = proteins extracted from healthy Datura plants infected with TMV virus and treated with performic acid.

The application of these proteins to pepper plants followed by an inoculation with the Tobacco mosaic virus did not cause any symptoms when compared to plants that were not inoculated with the virus. This suggests that the protein may function as an inhibitor during the early stages of the virus infection, preventing the virus

Table 1. The effect of Ganoderma extract on the Tobacco mosaic virus (TMV) percentage of multiplication

Ganoderma extract spraying	% of preventing the virus multiplication (inducing the resistance)	Absorption at 406 nanometers two weeks after the treatment
Infection with the virus after 3 days of	37a	0.612
Infection with the virus after 6 days of spraying	38a	0.598
Infection with the virus before 3 days of spraying	58b	0.404
Infection with the virus before 6 days of spraying	69c	0.296

*The averages that are denoted with similar letters for each column are not significantly different according to Duncan Multiple Range at a likelihood level of (0.05).

from replicating or causing damage to the viral capsid upon entry. Alternatively, the protein may work to block the virus's ability to attach to or enter cells by destroying the virus's available cell surface receptors, which would prevent the virus from attaching to or entering the cell. Consequently, the cells treated with these proteins will become more resistant to the virus. These findings corroborate those of Gera *et al.* (1986), who investigated tomatoes that are consistently infected with the tobacco mosaic virus and found that plants develop anti-viral principles (AVPs). The two researchers discovered that applying AVPs to plants prior to virus infection or combining them with the tobacco mosaic virus, which is used to infect tomatoes, reduced the intensity of the virus infection. The early phases of infection were when AVP production started, and as this production increased, the virus's concentration in the plant gradually dropped and its capacity to spread new infections reduced. Oversensitivity symptoms did not accompany the generation of AVPs. Furthermore, when the base halves of *Nicotiana glutinosa* plants were infected with either TNV or TMoV, compounds resembling AVPs were isolated from the non-infecting extreme halves of the plant leaves. The tomato plant that was systemically infected with TNV produced the highest amount of AVPs at 26°C, a temperature that is inappropriate for the virus's reproduction. Furthermore, it was discovered that the AVPs generated in plant tissue can affect the infection of the (TMoV) virus in those tissues by transferring to other tissues (Ready *et al.,* 1986).

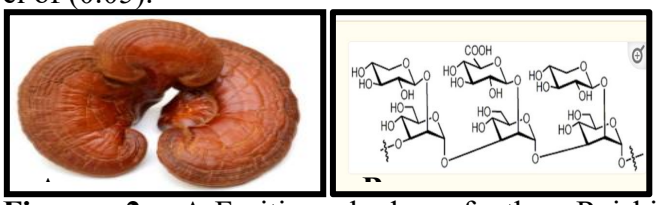

Figure 2. A-Fruiting body of the Reishi mushrooms (*Ganoderma lucidum).* B-Polysaccharide, compound Glucuronoxylomannan (GXM), quoted from (Mehta and Jandaik, 2012).

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These compounds are similar to AVPs and phytoalexins in many ways. They are both formed after the host and pathogen interact, and both are resistant species that can be infected, though the process is quicker in the resistant species. This was discovered when comparing tomato plants with tobacco mosaic virus in susceptible plants. When compared to species that are capable of infection to a level high enough to halt the pathogen's reproduction, the resisting species has a higher ultimate concentration of both of them (Sela and Applebaum,1962).

The viral infection intensity varies statistically significantly between average treatments, according to Table 1 results. The results of the ELISA test, which show a positive reaction indicated by the yellow color in the wells of ELISA plates containing the extracts of the pepper samples, indicate that the treatment of reishi mushrooms or *G. lucidum* resulted in the lowest severity of injury. The treatment with reishi

mushroom extract had the highest percentage of preventing the virus multiplication, 69%, after the plants were sprayed with the Ganoderma extract and six days before the plants were infected with the TMV virus. The wave absorption was 0.296 nm at a wavelength of 405 nm, as opposed to the treatment infected by the virus alone, which was 0.972 nm. On the other hand, the wave absorption value measured three days before to the virus infection by spraying the plants with Ganoderma extract was 0.404 nanometers, indicating a 58% protection rate against virus multiplication. Conversely, the wave absorption values following the application of Ganoderma extract to the plants 3 and 6 days post-infection were 0.612 and 0.598 nm, respectively, indicating a 37% and 38% reduction in virus multiplication.

The *Ganoderma lucidum* fungus is one of the basidiomycetes fungi that are characterized by their anti-microbial effect against various pathogens. This is because the fungus produces 400 effective compounds from its fruit body and mycelium, including anti-microbial activity. These compounds primarily consist of polysaccharides, amino acids, flavonoids, proteins, sterols, organic germanium, vitamins, minerals, and other essential elements like terpenoids (Kim and Kim, 1999; Monzingo *et al.,* 1993).

As a result of its highly effective compounds that block the virus's ability to infect plants, *Ganoderma lucidum* has been shown by Brandt and Piraino (2000) to be able to inhibit TMV infections with a percentage of 65-75%. These compounds also build barriers that stop the virus from infecting plant cells and demonstrate the plant's resilience to viral infection even at high temperatures. Compounds that have been demonstrated to be effective in this fungus include polysaccharides like glucuronoxylomannan (GXM), as presented in Figure 2.

By interfering with the virus's ability to adsorb and penetrate during its assembly and release, terpenoids function as antivirals at the membrane level. The Lignans impact the virus at the initial stages of infection, while the phenol compounds exhibit distinct roles over the entire virus life cycle. Peptides and proteins have significant effects on how closely the early stages of a virus infection overlap with one another as well as how much protein is created at that level.

Learn about this research and the potential to extract molecules with a protein nature from some plants. These chemicals have been shown to boost resistance to viral infections by inhibiting the growth of tobacco mosaic viruses. We suggest looking into additional compounds from other economically significant plants that may have the ability to stimulate resistance against pathogens, whether they be fungi or viruses, in pepper plants, in addition to the substances extracted from the leaves of datura and reishi mushrooms. In addition to being inexpensive, simple, and effective, they can be used as a substitute for environmentally hazardous chemical pesticides that are often used to control or prevent plant illnesses and injury occurs (Apablaza and Bernier,1972).

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