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Research Article

Potential of *Curcuma longa* Linn. (Turmeric) in management of *Callosobruchus chinensis* L.: *In-silico* analysis

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ABSTRACT: *Curcuma longa* Linn. (Turmeric) is a perennial herb known for its medicinal properties and is a common ingredient in Ayurvedic medicine, used for the prevention and treatment of several health disorders. Turmeric is also used as a common product in traditional pest management practices in India. As the pertinent problems associated with long-time exposure to chemical insecticides are becoming well known, the search for more eco-friendly alternatives has become necessary. In the present study, we aim to understand the efficiency of turmeric as a potent botanical against the common stored grain pest of legumes, *Callosobruchus chinensis*. Computer–based *in-silico* techniques are used as they allow a faster and more precise assessment of drug action. Direct toxicity tests are performed and the results are corroborated with the *in-silico* simulations to confirm the efficacy of turmeric as a potent botanical in management of *C. chinensis.*

KEYWORDS: Docking, Glutathione S-Transferase, pest, phytochemicals, thymidylate synthase

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INTRODUCTION

Curcuma longa Linn. is a perennial herb of the family Zingiberaceae commonly known as Turmeric or Haldi. It is cultivated throughout the Oriental region as a popular spice. Turmeric is traditionally used for the prevention and therapeutic treatment of diseases. It is also used as a potent antioxidant, anti-inflammatory, antimutagenic, antimicrobial and anticancer agent (Prasad and Agarwal, 2011). Due to all of these properties turmeric has been used in Ayurvedic medicine for centuries (Dudhatra *et al.,*2007). Apart from its multiple uses in medicine, turmeric is also found to have insecticidal properties against certain agricultural pests (Damalas, 2011). It has established its uses in traditional pest management practices (Gopi *et al*., 2016). Plant extracts are known to provide a safer, affordable and bio-degradable alternative to chemical insecticides (Prasanthi *et al*., 2017). So, the present study is designed to access the efficacy of turmeric in the management of *Callosobruchus chinenesis* using *in-silico* techniques.

Callosobruchus chinensis is a stored grain pest of legumes (Raina, 1970), primarily distributed in tropical Asia. It is also known as the adzuki bean weevil, Chinese bruchid, cowpea bruchid or pulse beetle. Every year it damages large amount of stored grains of green gram, lentil, cowpea, pigeon pea, chickpea and other pea species. The pest completes its larval development inside pods of legumes damaging the grains in the process. *Callosobruchus chinensis* thus cause serious qualitative and quantitative losses to stored legume products (Khairnar *et al.,* 1996).

In-silico approach in drug design increases the ability to predict and model the most relevant pharmacokinetic, metabolic and toxicity endpoints, thereby accelerating the drug discovery process (Waterbeemd and Gifford, 2003; Batool *et al.,* 2019). In-silico approaches have been applied to study the control of disease vectors such as mosquitoes (Elamathi *et al*., 2014). Similar *in-silico* techniques are used to target two enzymes of the stored grain pest, *Callosobruchus chinensis.* Glutathione S-Transferase is a family of metabolic isozymes that catalyzes the conjugation of Glutathione to a wide variety of exogenous substrates. This makes the compounds more water soluble thus helping in detoxification (Eaton and Bammler, 1999). The compounds targeted by Glutathione

S-Transferase includes a diverse range of exogenous toxins including pesticides. Thus, it can be postulated that if the function of the enzyme Glutathione S Transferase is suppressed then the natural detoxification power of the insect will be hampered. In such a condition if a phytochemical is used to kill the insects, there will be higher chances for it to be effective as a major pathway of detoxification has been inactivated. Thymidylate synthase on the other hand, plays a key role in DNA synthesis (Liu *et al*., 2002). It catalyzes the conversion of Deoxyuridine Monophosphate (dUMP) to Deoxythymidine Monophosphate (dTMP). Deoxyuridine Monophosphate (dUMP) undergoes reductive methylation with N5, and N10-methylene tetrahydrofolate to form dTMP yielding dihydrofolate as a secondary product.

REACTION

5,10-methylenetetrahydrofolate + dUMP $\xrightarrow{\text{Thymidylate Synthase}}$ Dihydrofolate + dTMP

This is the sole de novo pathway for the production of dTMP. Thymidylate synthase is the only enzyme in folate metabolism by which the 5,10-methylenetetrahydrofolate is oxidized during one-carbon transfer (Costi *et al*., 2002). Thus, the enzyme is essential for regulating the balanced supply of the four DNA precursors for the formation of normal DNA. Defects in the enzyme activity will affect the process of DNA synthesis causing a number of several biological and genetic abnormalities.

The *in-silico* analysis is followed by direct toxicity tests to ascertain the effect of *Curcuma longa* as a natural control agent of stored grain pest *Callosobruchus chinensis*. In addition to insect mortality, the effect of the test extract on oviposition and the per cent emergence of adult insects are also studied to obtain a comprehensive result.

MATERIALS AND METHODS

Preparation of receptor and ligand

The molecular structures of the receptors (Figure 1), Glutathione S-Transferase (1GNW) and Thymidylate Synthase (1TSM) were downloaded from PDB database (https://www.rcsb.org/). RCBS PDB (Research Collaborators for structural Bioinformatics Protein Data Bank) database organizes 3-D structural data of large biological molecules including proteins and nucleic acids of all the organisms through experimental methods (Parasuraman, 2012).

The major phytochemicals present in turmeric include curcumin, demethoxycurcumin, bisdemethoxycurcumin, turmerone, ar-tumerone, curlone (β-turmerone), curcuphenol among others (Dosoky and Setzer, 2018, Nelson *et al*., 2017). Two reference ligands were also selected based on their activity against the target receptors. Ethacrynic acid, a diuretic drug commonly used as an inhibitor of Glutathione S-Transferase and 5-fluorouracil, an inhibitor of Thymidylate synthase, were selected (Van Triest *et al.,* 2000; Awasthi *et al.,* 1993). The structures of these ligands (Figure 2) were downloaded from Zinc database (https://zinc.docking.org/) which is a free database of commercially available compounds especially prepared for virtual screening. The vendor catalog TCM Database@ Taiwan was used which is the world's largest traditional

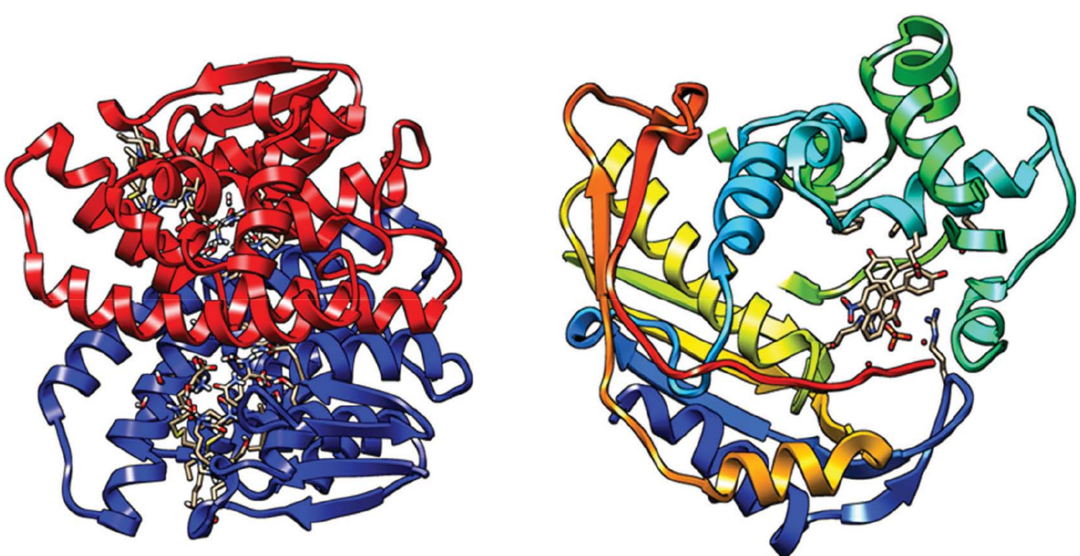

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Figure 1. Structures of the receptors, Glutathione S-Transferase (1GNW) and Thymidylate Synthase (1TSM).

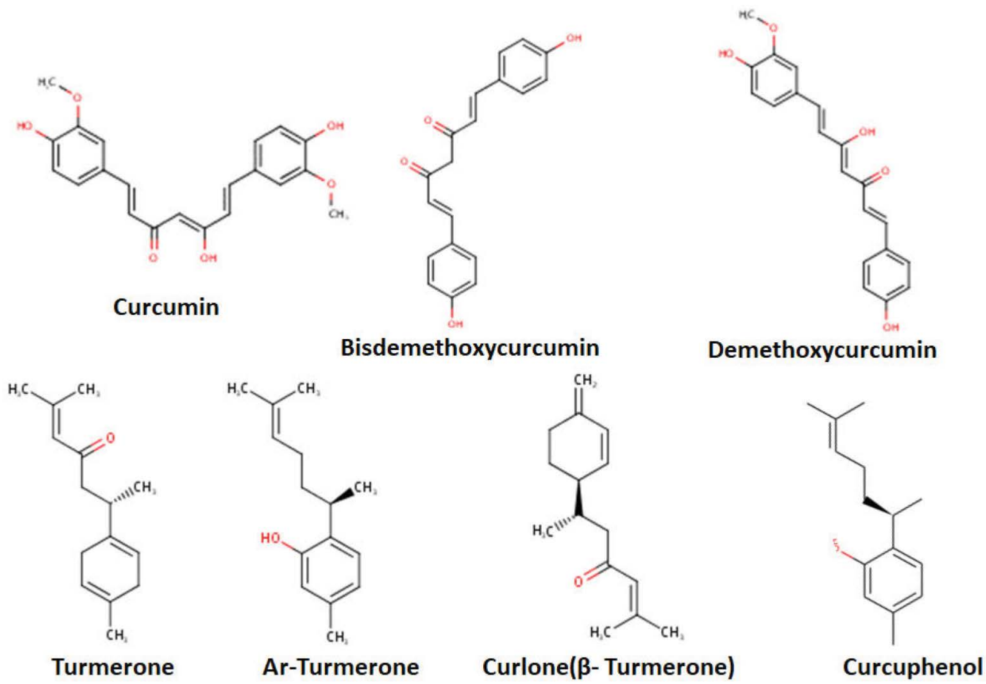

Figure 2. Structures of phytochemicals present in Turmeric.

Chinese medicine database for *in-silico* drug screening (Calvin and Chian, 2011).

Drug likeness prediction

The Lipinsky's rule of 5 was used to determine if the selected ligand molecules with specific pharmacological or biological activity have the physical and chemical properties to make them active drugs. The rule was formulated by Christopher A. Lipinski in 1997. SwissADME web tool provides a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry (Daina *et al*., 2017). The web tool is freely accessible (http://www.swissadme.ch) and provides computed parameters that would make a molecule affective as a drug.

Pharmacokinetics

Pharmacokinetics describes the interaction of a drug with an organism's biological system and includes the administration pathway of the drug, its absorption into the blood stream, its distribution and metabolism in the body tissues and finally its excretion out from the body. Pharmacokinetic properties are also affected by the route of administration and the dose of the administered drug (Benet and Zia-Amirhosseini, 1995). Pharmacokinetics of the ligand molecules were obtained from the SwissADME webserver and analyzed to determine the fate of the compounds once they are administered into the organism.

Molecular docking

Molecular docking is a popular tool in drug design (Ruyck *et al*., 2016). The possible molecular interactions between the receptors and the ligand molecules were predicted by using docking software, Molegro Virtual Docker (MVD 2010.4.0) for Windows. Molegro Virtual Docker is an integrated platform for predicting protein-ligand interactions that handles all aspects of the docking process from preparation of the molecules for to prediction of the binding modes of the ligands (Thomsen and Christensen, 2006). The identification of ligand binding modes was done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule. The highest scoring solutions are further analyzed using a scoring function that correctly ranks candidate dockings.

Visualizing receptor-ligand interactions

Visualization is an important aspect in the molecular modeling studies. BIOVIA Discovery Studio Visualizer is a free molecular modeling application used for viewing, sharing and analyzing protein and small molecule data developed by Dassault Systems BIOVIA 2021 (Irwin and Shoichet, 2005).

Toxicity tests using turmeric extract

In order to reach conclusive outcome toxicity tests were performed. Adult beetles of *Callosobruchus chinensis* were collected and maintained on healthy seeds of green gram (*Vigna radiata*). The insects were kept in plastic containers (12 cm x 8 cm) with their mouth tied with muslin cloth at room temperature (18 - 32 °C) and 60-90 % relative humidity. *Curcuma longa* samples were collected and washed, then dried in room temperature for 6 to 10 days. The samples are than macerated and subjected to methanolic extraction using a Soxhlet apparatus for 15 to 18 hours. Soxhlet extraction is a less time-consuming method with higher extraction efficiency (Zhang *et al.,* 2017). The methanolic extracts are than dried using a rotary evaporator and bottled for experimental use.

For the toxicity test, insects were first immobilized and kept in petri dishes. Then 10-20 microliter solution of different concentrations (2%, 4%, 6%, 8%, 10%, 12%) of plant extract were applied on the dorsal side of each insect with the help of micropipette. After insects were examined continuously and those that did not move or respond to gentle touch were considered dead. Insect mortalities were recorded at 24, 48 and 72 Hours After Treatment (HAT) (Paramasivam and Selvi, 2017; Sun, 1963).

Additionally, the effect of plant extract on oviposition and emergence of adults was studied. For this purpose, one pair of newly emerged insects were introduced in a chamber and allowed to mate. They were fed on the green gram seeds mixed with varying concentrations of plant extracts. To tabulate the effect on oviposition, the adults are removed from the mating chamber after 8 days and number of eggs laid are counted (Kalita, 2016). For emergence of adult the mating chambers with eggs are kept undisturbed for 15-20 days and the number of adult emerged were counted until there was no emergence of adult for 3 consecutive days.

The data thus obtained is analyzed using R software (R Core Team (2020). The Probit analysis was done using the Henry simplified table and following the protocol of Finney (1971). The table and graph preparation and SD value measurement were done using Microsoft excel, 2016.

RESULTS

Computer based molecular docking techniques were used to interpret the effectiveness of turmeric in the management of pest species *Callosobruchus chinenesis*. The phytochemicals present in turmeric were identified through literature review and these chemicals were then used to prepare protein-ligand interaction models in silico. For this purpose, at first, their physiochemical properties and pharmacokinetics were analyzed (Tables 1 and 2) to understand the fate of these chemicals inside the body of an organism

The receptor and ligand molecules were downloaded from online databases in compatible formats. Then the 3D

Table 1. Physiochemical properties of selected ligands

Sl. No	ZINC Id	Name of ligand	Physiochemical properties									
			Formula	**Molecular weight (gm/mol)**	**No. of heavy atoms**	**No. of arom. Heavy atoms**	**Fraction Csp3**	**No. of rotatable bonds**	**No. of H-bond acceptors**	**No. of H-bond donors**	**Molar Refractivity**	**TPSA (A)**
1	ZINC000100067274	Curcumin	$C_{21}H_{20}O_6$	368.38	27	12	0.1	7	6	3	103.7	96.22
2	ZINC000100191509	Demethoxycurcumin	$C_{20}H_{18}O_5$	338.35	25	12	0.05	6	5	3	97.21	86.99
3	ZINC000001651126	Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	308.33	23	12	0.05	6	4	2	89.82	74.6
4	ZINC000013377636	Turmerone	$C_{15}H_{22}O$	218.33	16	0	0.53	4	1	0	70.88	17.07
5	ZINC000006071066	ar-Turmerone	$C_{15}H_{20}O$	216.32	16	6	0.4	4	1	0	69.75	17.07
6	ZINC000003645382	Curlone (β-Turmerone)	$C_{15}H_{22}O$	218.33	16	0	0.53	4	1	0	70.88	17.07
7	ZINC000001616758	Curcuphenol	$C_{15}H_{22}O$	218.33	16	6	0.47	4	1	1	71.57	20.23

Table 2. Pharmacokinetics of selected ligands

Sl. No	ZINC Id	Name of ligand	Pharmacokinetics								
			GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (skin permeation) (cm/s)
1	ZINC000100067274	Curcumin	HIGH	NO	NO	NO	NO	YES	NO	YES	−5.72
2	ZINC000100191509	Demethoxycurcumin	HIGH	NO	NO	YES	NO	YES	NO	YES	−5.52
3	ZINC000001651126	Bisdemethoxycurcumin	HIGH	YES	NO	YES	NO	YES	NO	YES	−5.87
4	ZINC000013377636	Turmerone	HIGH	YES	NO	NO	NO	YES	NO	NO	−5.27
5	ZINC000006071066	ar-Turmerone	HIGH	YES	NO	NO	NO	NO	NO	NO	−4.79
6	ZINC000003645382	Curlone (β-Turmerone)	HIGH	YES	NO	NO	YES	YES	NO	NO	−4.78
7	ZINC000001616758	Curcuphenol	HIGH	YES	NO	NO	NO	NO	YES	NO	−3.89

structures of the macromolecular targets were analyzed to identify key sites of interactions that are essential for their biological activity. This information was used to study the interactions of the target enzymes with the ligand molecules that would interrupt the biological pathways essential for survival of the organism (Wenbo and Mackerell, 2010). After preparation of the receptor and ligand molecules molecular docking was performed to predict protein-ligand interactions. For the purpose a fully functional trial version of Molegro Virtual Docker (MVD) was used. The software predicts binding modes of the ligands and estimates their energy of interactions with the receptor. Both the receptor and ligand molecules were imported into the software's interface and the docking wizard was run. The results of docking were displayed as docking scores. The mean docking scores of all the ligands were calculated. Then the results were presented as bar diagrams (Figure 3 and Figure 4).

Based on the docking scores, the protein-ligand interactions were studied. Using Discovery Studio Visualizer, the interaction of ligand with the active site of the target proteins were visualized in 3D. The different types of interactions of the ligands with the amino acid residues of the target proteins were observed in 2D (Figures 5 and 6).

To further establish the results of the *in-silico* analysis, laboratory experiments were conducted. Direct toxicity tests were performed using methanolic extract of turmeric. The results indicate that the mortality of the target insect increased with increase in concentration of the test extract. At 2%, 4%, 6%, 8%, 10% and 12% concentration of test extract, the percentage mortality of insect was 28.3%, 36.65%, 43.3%, 53.3%, 61.65% and 75% respectively (Table 3). The change in percentage mortality with respect to the change in test concentration of extracts are indicated in a line graph (Figure 7). The results thus clearly indicate the viability of turmeric extract as a control agent for pest species *Callosobruchus chinensis.* The effect of test extract on the oviposition and emergence of adults was also analyzed. The effect of test extract on the average number of eggs laid was found to be 33, 26, 23.3, 18.6, 12.33, 10 at 2%, 4%, 6%, 8%, 10% and 12% concentration of doses, respectively (Table 4). The effect of turmeric extract on the average number of adults emerged from eggs was found to be 22.33, 16.66, 10.33, 9.66, 5.33, 5.66 at 2%, 4%, 6%, 8%, 10% and 12% concentration of doses, respectively (Table 5). The effect of test extract on the oviposition and emergence of adult insects with respect to the change in concentration of the test extract are shown through line diagrams (Figure 8 and Figure 9). In all the parameters taken in the study, the results obtained from the treatment was significantly higher than the control.

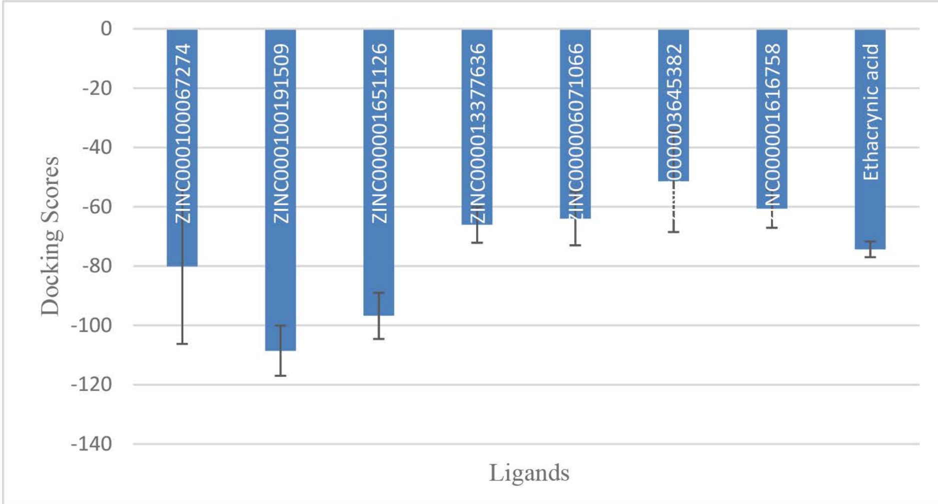

Figure 3. Graph representing docking scores of ligands against Glutathione S-Transferase. Etharcrynic acid was used as reference ligand during docking simulation.

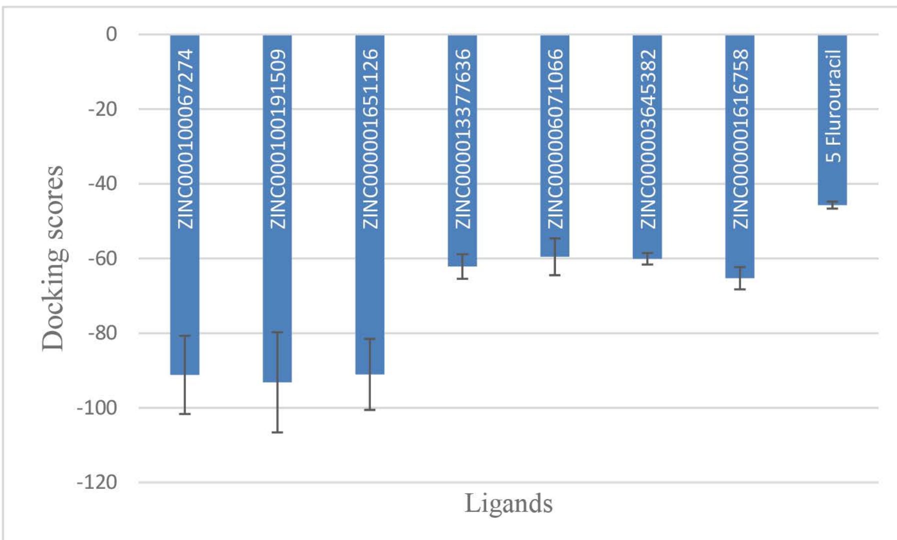

Figure 4. Graph representing docking scores of ligands against Thymidylate Synthase. 5-Fluorouracil was used as reference ligand during docking simulation.

DISCUSSION

According to the Food and Agricultural Organization of the United Nations, around 811 million people around the world grappled with hunger in the year 2020. A global pandemic, climate change and international conflicts are only some of the problems that have major impact in food security. In this scenario, no country in the world can afford to compromise its food resources and loose stored products to pest infestations (Stathers, *et. al.* 2019). Chemical pesticides have been used for years but their long-term impact on human health as well as the environment involved has also been extensively documented (Nicolopoulou-Stamati *et al.,* 2016). In the present situation an environment friendly and eco-friendly management of insect pests is required. Turmeric has proven therapeutic effects and also has documented uses as an insecticide. In the present study turmeric extract is used to control the common stored grain pest of pulses, *Callosobruchus chinensis.* The study aims to find the effect of turmeric as a management tool of the pest species. The experimental process begins with an *in-silico* analysis aimed at accessing the effect of phytochemicals present in turmeric with two target enzymatic receptors in the pest, through computer simulations. Similar techniques have been applied against several insect receptors (Yadav *et al.,* 2015) as well as in pharmaceuticals for drug discovery (Ekins *et al.,* 2007). Computer based techniques facilitates precise and streamlined experimental setups. The results of the *in-silico* analysis clearly indicate that the selected phytochemicals of turmeric effectively interact with the

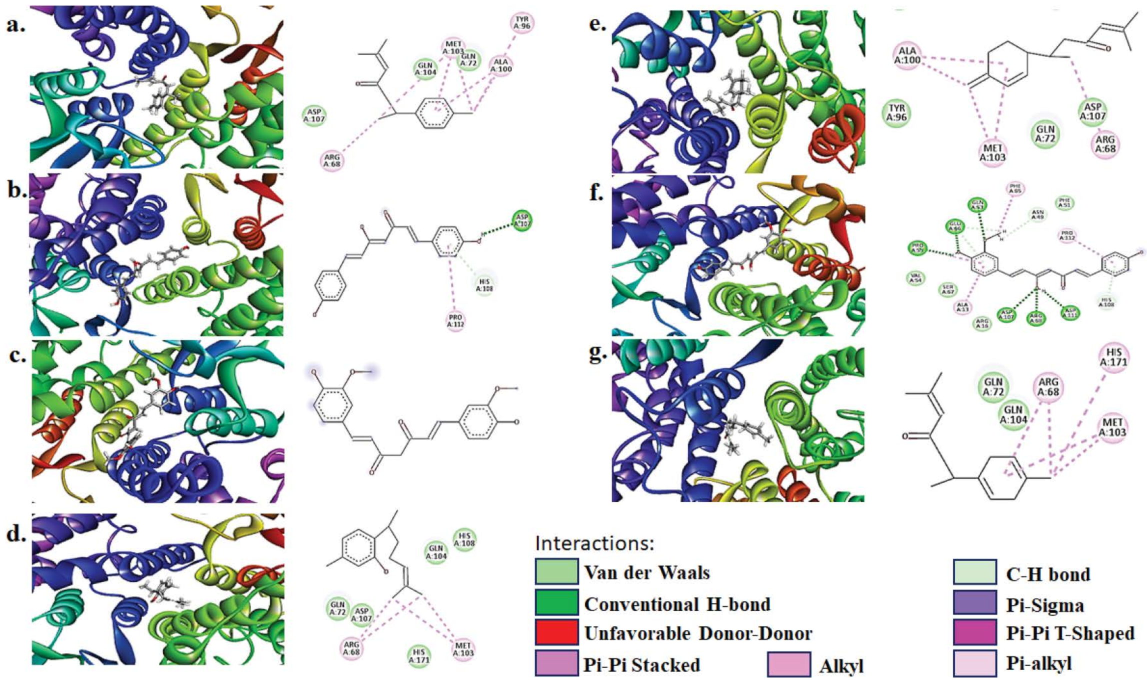

Figure 5. Interactions of ligands with the enzyme Glutathione S-Transferase displayed in 3D and 2D, **a.** Ar-turmerone, **b.** Bis-demethoxy-curcumin, **c.** Curcumin, **d.** Curcuphenol, **e.** Curlone, **f.** Demethoxycurcumin and **g.** Turmerone.

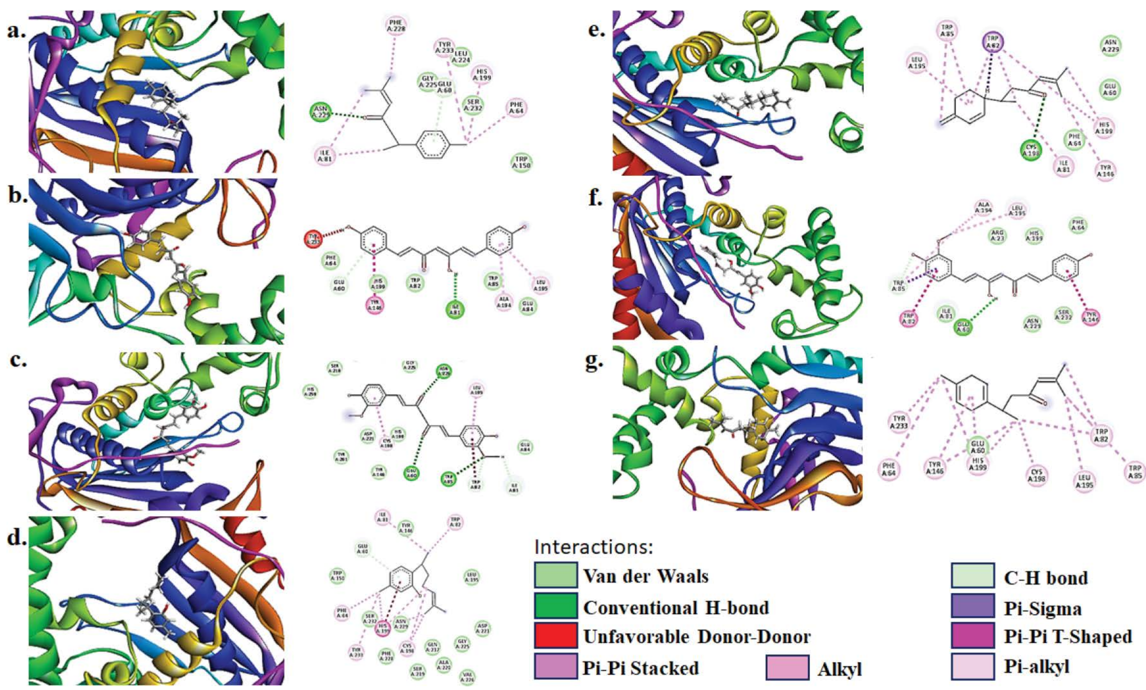

Figure 6. Interactions of ligands with the enzyme Thymidylate Synthase displayed in 3D and 2D mode, **a.** Ar-turmerone, **b.** Bis-demethox-ycurcumin, **c.** Curcumin, **d.** Curcuphenol, **e.** Curlone, **f.** Demethoxycurcumin and **g.** Turmerone.

selected enzymes of the target pest and inhibit their biological function. Based on the metabolic functions of the enzymes used, it is established that the phytochemicals hamper the life cycle of the target pest. To concur the results of the *in-silico* analysis, laboratory experiments were performed. Direct toxicity test performed using methanolic extracts of turmeric showed a dose dependent increase in mortality of the target pest. The effect of the test extract on oviposition and emergence of adults from eggs were also studied. Both the additional parameters showed dose dependent increase in their efficacy. The results thus obtained can be correlated with results of Tripathi *et al.,* 2009; Singh *et al.,* 2017 and

Table 3. Mortality of *Callosobruchus chinensis* after treating with extract of *Curcuma longa*

Sl.No.	Plant Extract	Total no. of Dead Insects					
		Conc. of Doses					
		2%	4%	6%	8%	10%	12%
1	*Curcuma longa*	28.3±0.57	36.65±0.57	43.3±0.57	53.3±1.15	61.65±0.57	75±1
2	Control	0±0	0±0	0±0	1.65±0.57	0±0	1.65±0.57

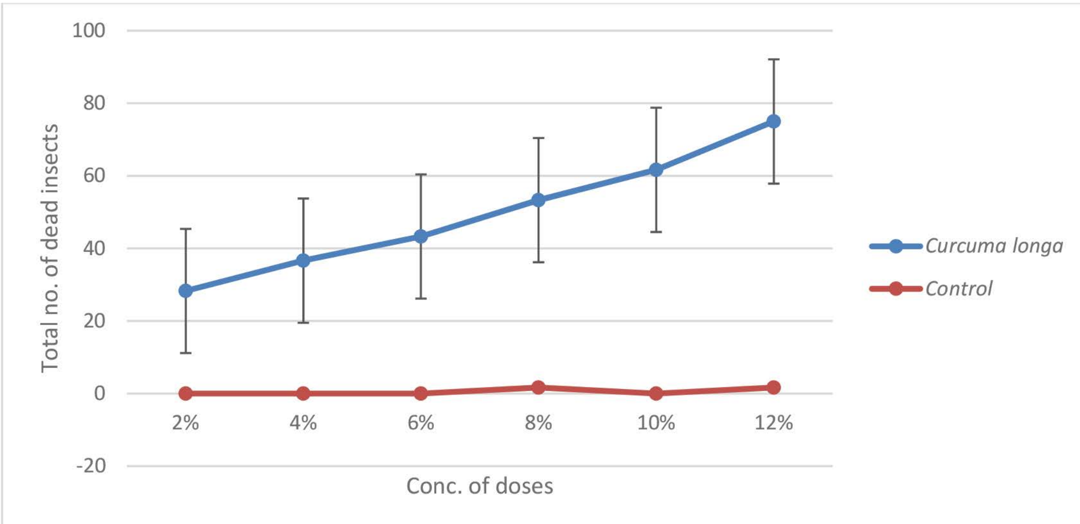

Figure 7. Graph indicating mortality of target insects on treatment with different concentrations of turmeric extract.

Table 4. Effect of turmeric extract on the oviposition of *Callosobruchus chinensis*

Sl. No.	Plant Extract	Total no. of eggs laid					
		Doses					
		2%	4%	6%	8%	10%	12%
1	*Curcuma longa*	33±5	26±5.56	23.33±4.72	18.66±4.04	12.33±2.51	10±2
2	Control	69±1	67±5	67.33±4.72	66.66±5.50	66±7	65.66±6.11

Table 5. Effect of turmeric extract on the emergence of insects of *Callosobruchus chinensis*

Sl.No.	Plant Extract	Total no. of adult emerged					
		Conc. of Doses					
		2%	4%	6%	8%	10%	12%
1	*Curcuma longa*	22.33±5.50	16.66±5.03	10.33±2.08	9.66±2.08	5.33±1.15	5.66±0.57
2	Control	52.33±3.05	58.66±3.51	54.33±3.51	47.33±3.21	45±2.64	53±5.56

Nisha and Chand 2019 which all suggest the efficacy of turmeric extract as a potent botanical in the management of *Callosobruchus* sp. The results of the study thus clearly indicate the effectiveness of turmeric extract as a control agent of stored grain pest *Callosobruchus chinensis.* Among all components of turmeric only curcumin does show potential inhibitory effects towards Glutathione isoenzymes (Appiah-Opong *et al.,* 2009). However, the lethal dose of curcumin has not been defined and can be considered to be much higher that the doses used for insect control. Apart from that, curcumin has additional properties as an oxygen radical scavenger, antioxidant properties achieved through modulation of glutathione levels, and anti-inflammatory

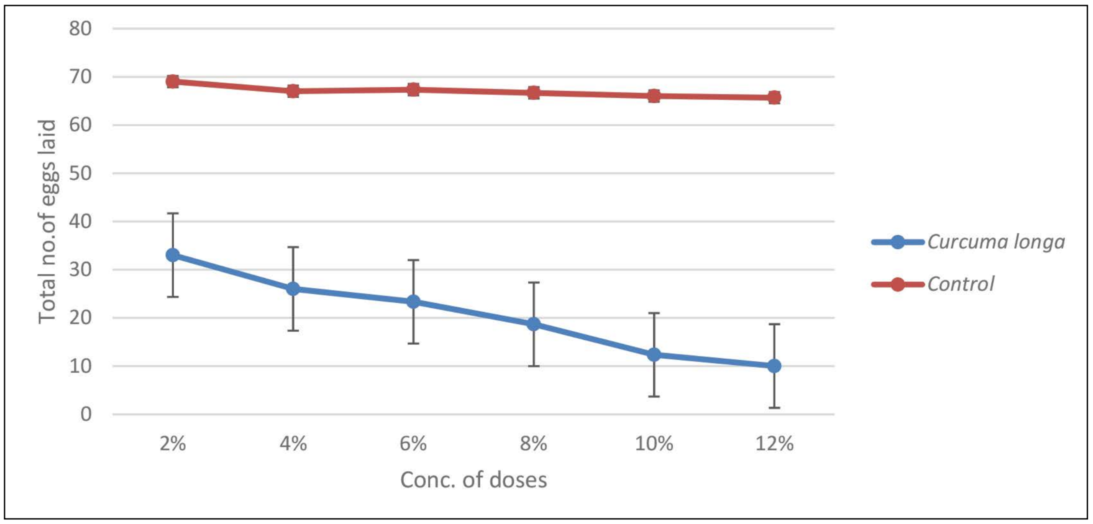

Figure 8. Graph indicating the effect of turmeric extract on the ovipostion of target insects.

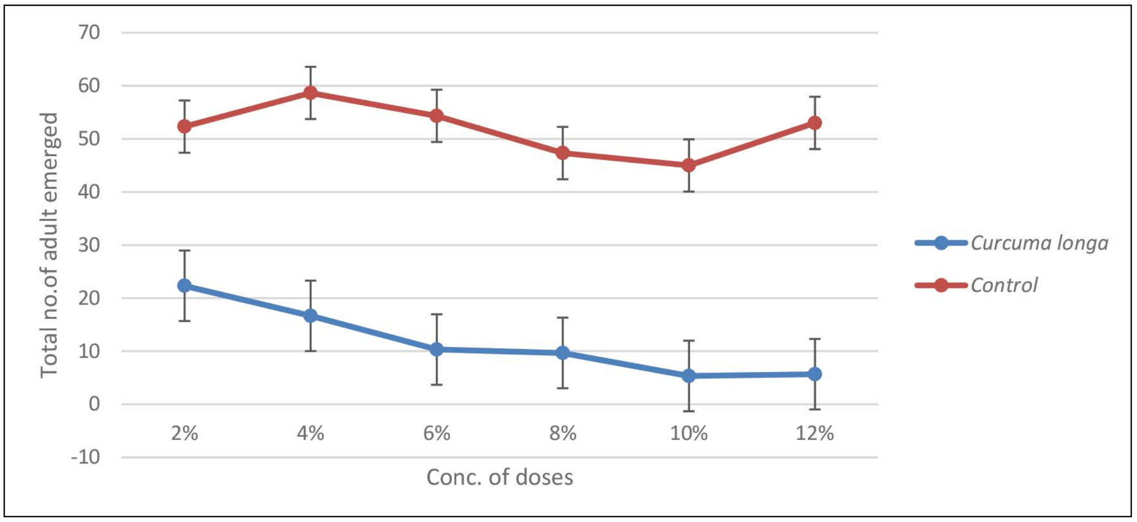

Figure 9. Graph indicating the effect of turmeric extract on the emergence of adults of target insects.

agent through inhibition of IL-8 release in lung cells (Saibal *et al.,* 2005). Thus, the susceptibility of cells to damage by curcumin can be somewhat compensated. Additionally, turmeric has been used as a therapeutic agent for ages and has been extensively studied for it's antibacterial, anti-inflammatory, antimicrobial, and anti-cancer effects (Hay *et al.,* 2019).

CONCLUSION

An *in-silico* approach was adopted to study the effect of *Curcuma longa* Linn. in the management of pest species *Callosobruchus chinensis.* For the study, the phytochemicals present in turmeric were used as ligands and their interactions with the target enzymes of the pest were analyzed. Two enzymes of the pest were targeted, Glutathione S-Transferase and Thymidylate Synthase. Both the enzymes are known to catalyze important biochemical reactions in the body of the organism. Docking simulations are performed to analyze the interactions of the plant phytochemicals of turmeric with the enzymes of the target pest. The results indicated that almost all the selected phytochemicals showed binding interactions with the target enzymes. So, they are capable of inhibiting the enzyme action in the target species. In order to verify the results of the *in-silico* study a toxicity assay was performed with extract of *Curcuma longa* against *Callosobruchus chinenesis.* The results of the toxicity assay clearly indicated that turmeric extract was effective in the natural control of the target insect. The test extract was also found to be effective in the control of oviposition and percent emergence of adult insects. Turmeric extract was found to be effective in control of adult insects and also hindered the reproductive efficiency of the insect species. Thus, the results obtained from study clearly indicates that *Curcuma longa* extract can be used as a potent botanical agent for the control of stored grain pest *Callosobruchus chinensis.*

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