1. INTRODUCTION

The tea plant *Camellia sinensis* (L.) O. Kuntze of family Theaceae is the oldest crop used in the preparation of non-alcoholic caffeine containing beverage. Tea has attractive aroma, pleasant odour and numerous medicinal benefits, and has been habitually consumed by people since 3000 B.C. Nutritional and therapeutic importance of tea arise from its unique combination of large number of constituents such as proteins, carbohydrates, amino acids, lipids, vitamins, minerals, alkaloids and polyphenols (Krishnamoorthy, 1987). Tea polyphenols specifically include several type of catechins compounds including catechin, epicatechin, epigallocatechin, gallocatechin, epigallocatechin gallate and epicatechingallate, flavanones and phenolic acids (Pan et al., 2003). Catechin present in tea possesses strong antioxidant activity especially in free radical scavanger acting (oxysinglet, triplet, hydroxy super hydroxyl) and chelating to prevent catalyst effect of metal ion in oxidative reactions (Mira et al, 2002; Gupta et al, 2002; Hara 2001; Weisburger 2000 and Catherine et al., 1996).

India has unique distinction of being the largest producer and consumer of tea in the world. Indian tea industry produces 1000 million kg tea from an area of 5,78,458 hectares (http://teaboard.gov.in/pdf/statgrowers). Tea industry in India has an annual turnover of rupees 8 million USD (http://www.siligurionline.com/industry/tea.htm) and provides employment to million people. In Himachal Pradesh, tea occupies about 2,063 hectares with annual production of 0.9 million kg (Tea Board of India, 2009) and thus contributes 0.01 per cent of the country’s tea production. A manifold increase in production of tea in India is mainly attributed to efficient and integrated agricultural practices including efficient weed management practices.

Weeds compete with crops for nutrient, sunshine and moisture. Besides reducing the yield, weeds also have adverse effects on tea viz. restricted branching, frame development in young tea, reduce plucking efficiency, harbour and serve as alternate host for many organisms including some important pests of tea. Depending upon the intensity of weed growth, extent of competition and weed species, weeds reduce the productivity
of tea by 10-50 per cent (Rao et al. 1977). Thus, all these situations make it necessary to rely on herbicides for an effective and timely weed control. In tea plantations, use of herbicides as a tool for controlling weeds is very much popular and have been widely used ever since their introduction—primarily due to their cost-effectiveness, efficiency in controlling diverse weed flora and less labour intensiveness etc.

Glyphosate [N-(phosphonomethyl)glycine], a member of organophosphorus family is a recommended herbicide for weed control in tea (Rahman, 1991). It is a systemic herbicide that is absorbed by foliage and then translocated throughout the plant via phloem. Once a herbicide molecule finds it place in living tissue of plant, a number of biochemical changes begin to take place. While herbicide molecule alters the metabolism of the plant, the latter degrades the structure and activity of herbicide by various biochemical processes. Depending upon the altered nature of herbicide metabolites in the plants, the growth and development of crop plant is affected in terms of both quality and quantity. Glyphosate has been proven as a potent and specific 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) enzyme inhibitor. EPSPS is a key enzyme of shikimate pathway.

Shikimate pathway one of the major biosynthetic pathway in plants (illustrated in Fig. 1.1) is localized in plastids of the all plant tissue types, including non-green tissues or most roots (Hermann and Beaver, 1999). This pathway consists of seven enzymatic steps and ends with the formation of chorismate, the branch point precursor for the biosynthesis of the aromatic amino acids tyrosine, tryptophan and phenylalanine (Haslam 1974; Weiss and Edwards 1980 and Conn 1986) and many other compounds such as P-aminobenzoic acids, ubiquinones and vitamin K. Approximately 20 per cent of the carbon fixed by plants is normally channeled through the shikimic acid pathway (Haslam 1993). Inhibition of EPSPS synthase deregulates the pathway which results in uncontrolled flow of carbon and the subsequent massive accumulation of shikimate or shikimate-3-phosphate in affected plant tissues. Accumulation of shikimate results in increased production of derived shikimate benzoic acids, such as protocatechuate and gallate acid in plants.
In the shikimate pathway third and fourth steps of the pathway are catalyzed by the bifunctional enzyme 3-dehydroquinate dehydratase (DHD, EC 4.2.1.10) / shikimate dehydrogenase (SDH, EC 1.1.1.25) that catalyses dehydration of 3-dehydroquinate to 3-dehydroshikimate and the reversible reduction of 3-dehydroshikimate to shikimate. Another step is the reversible formation of 5-enolpyruvylshikimate-3-phosphate (EPSP) and inorganic phosphate from shikimate-3-phosphate and phosphoenol pyruvate. The reaction is catalyzed by EPSP synthase (EC 2.5.1.19). Phenylpropanoid biosynthetic pathway is linked to shikimate pathway which is also an important metabolite route due to its role in the synthesis of phenolic compounds and a wide range of secondary plant products. At the starting point of phenylpropanoid pathway phenylalanine ammonia lyase (PAL EC 4.3.1.5) catalyze the elimination of ammonia from the phenylalanine to provide cinnamate interface with the shikimate pathway and regulate the production of phenolic compounds (Boerjan et al. 2003).
Glyphosate, a phloem-mobile herbicide is distributed symplastically along with other contents of sieve elements (Gougler and Geiger 1981; Kleier 1988; Lichtner 1984; Neuman et al. 1985 and Tyree et al. 1979). The phytotoxic action of a herbicide may affect one or more of the processes that maintain or regulate translocation and distribution of carbon among sinks, and thereby affect its phloem-mediated distribution. Toxic effect of herbicide on photosynthetic carbon fixation, allocation of both newly fixed and stored carbon, short-distance transfer and membrane transport by sieve elements interfere with export of both assimilated carbon and the herbicide. It binds with 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) thereby inhibiting plant amino acid biosynthesis (Franz et al., 1997). EPSPS is the only known enzyme target of glyphosate (Siehl, 1997). It affects many physico-chemical and physiological processes (Cole, 1985) Glyphosate molecule binds outside the EPSPS active site unavailable to phosphoenol pyruvate (PEP) (Sikorski and Gruys, 1997). It induces reduction in photosynthesis, degradation of chlorophylls, inhibition of auxin transport and enhancement of auxin oxidation. These aspect of mode of action are either a direct consequence of the blockage of shikimate pathway or as a result of feedback mechanisms (Singh et al. 1991).

Binding of glyphosate EPSPS has been shown to be competitive with phosphoenol pyruvate (PEP) and uncompetitive with respect to shikimate-3-phosphate (Dodge 1989). The above details of shikimic acid biosynthetic pathway and mode of action of glyphosate via inhibition of this pathway are indicative of the fact that major biochemical quality attributes directly or indirectly are part of this pathway and may get affected adversely by glyphosate application leading to quality deterioration of tea.

At world level, among different plant protection chemicals consumed in agriculture, herbicides top the list. Off late, the use of herbicides in India is on the rise and increased use may pose opportunity for drift of chemicals on non-target areas also and therefore it is imperative to work out the risk of environmental contamination including farm produce quality after field application of the chemicals. Glyphosate [N-(phosphonomethyl)glycine] a world widely used herbicide in non-cropped and cropped situations including tea plantations, vineyards and roadsides. Even though the herbicide
application is target specific but there is always a possibility of drift of chemical on non-target plants also. Therefore, an important consideration in chemical weed control is degradation of herbicide in soil and its residues in crop produce.

The degradation processes of chemicals occurs at various rates that depend on type of compound and matrix in which it is present, as well as on environmental factors. Glyphosate, an organophosphorus group based herbicide, on degradation it yields AMPA (aminomethyl phosphonic acid) a major metabolite of glyphosate which finally degraded and mineralized to CO$_2$ (Fig 1.2).

![Degradation of glyphosate](image)

Degradation of glyphosate occurs under aerobic and anaerobic conditions in soils and in sediments (Rueppel et al. 1977; WHO 1994). Glyphosate adsorption to soil occurs through the phosphoric acids moiety in its phosphonate anion form as phosphate does in soil (Sprankle et al. 1975b). Glyphosate has a high water solubility (11.6 g L$^{-1}$) and low hydrophobicity (log $K_{OW}$ = -4.1) therefore it strongly sorbs to soil with sorption coefficients values greater than 1.000 L kg$^{-1}$ (Cheah et al. 1998). Glyphosate is potentially degraded by microorganisms in the soil solution. Bioavailability of herbicide
molecules to microorganisms is normally lower when these molecules are sorbed than when they are present in the soil solution. As a result of high sorption rate and the strong soil retention, it can persist in the soil sorbed in its original form as a bound residue. Therefore, the degradation of glyphosate in soil and in edible plant parts is an essential component to determine the herbicide activity and its effects on quality.

Keeping in view the above facts and moreover there is no information available on the effect of glyphosate on biochemical constituents of tea and its degradation behaviour. Therefore, the present investigation entitled, “Studies on effect of glyphosate on biochemical attributes of tea [Camellia sinensis (L.) O. Kuntze]” was undertaken during 2008 and 2009 with the following objectives:

(i) To study the effect of glyphosate on biochemical attributes of tea

(ii) to investigate the degradation behaviour of glyphosate.